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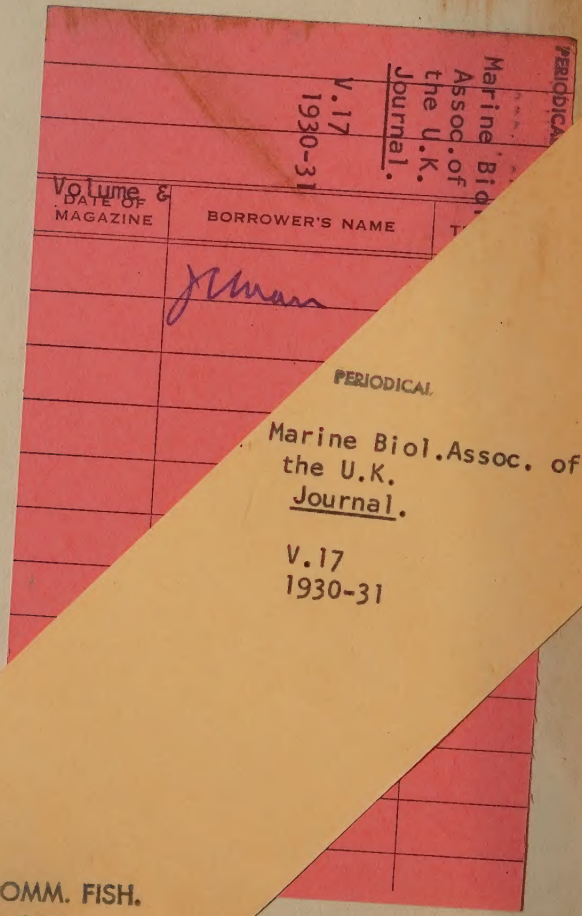

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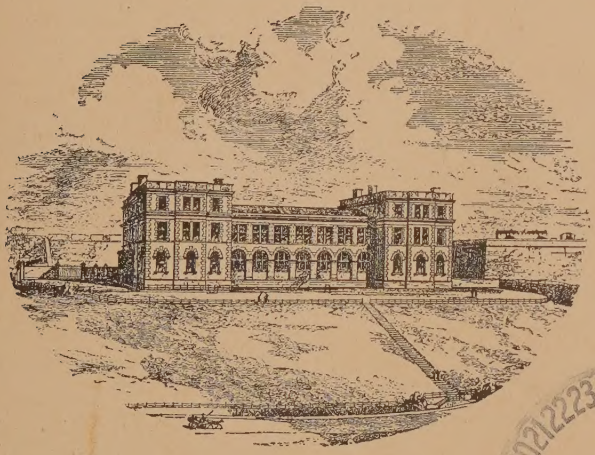

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CONTENTS OF VOLUME XVII.

(NEW SERIES.)

Report of the Council— PAGE

1930 587

Balance Sheet—

1930–31 604

List of Annual Subscriptions paid during the Year: 1st April, 1930, to 31st March, 1931 607

List of Donations 612

AMIRTHALINGAM, C.

See ORTON, J. H.

ATKINS, D.

On Abnormal Conditions of the Gills in *Mytilus edulis*. Part II. Structural Abnormalities, with a Note on the Method of Division of the Mantle Cavity in Normal Individuals 489

Note on some Abnormalities of Labial Palps and Foot of *Mytilus edulis* . . 545

Note on the Regeneration of the Gill of *Mytilus edulis* 551

ATKINS, W. R. G.

The Deterioration of Fabrics Exposed on a Roof after Treatment with Fishing-net Preservatives 473

Note on the Condition of the Water in a Marine Aquarium 479

ATKINS, W. R. G., AND POOLE, H. H.

A Preliminary Comparison of the Neon Lamp and Potentiometer Methods of Submarine Photo-Electric Photometry 617

See also GALL, D. C.

BERRILL, N. J.

The Natural History of *Bulla hydatis* Linn. 567

BORDEN, M. A.

A Study of the Respiration and of the Function of Hæmoglobin in *Planorbis corneus* and *Arenicola marina* 709

BORLEY, H. J. H.

Some Additions to the Sponge Fauna of Plymouth 839

CARRUTHERS, J. N.

Further Investigations upon the Water Movements in the English Channel. Drift-Bottle Experiments in the Summers of 1927, 1928 and 1929, with Critical Notes on Drift-Bottle Experiments in General 241

CONTENTS OF VOLUME XVII.

CHOUCROUN, N. PAGE

On the Hypothesis of Mitogenetic Radiation 65

DAWES, B.

The Absorption of Fats and Lipoids in the Plaice (*P. platessa* L.) . . . 75

Growth and Maintenance in the Plaice (*Pleuronectes platessa*). Part I . . 103

Growth and Maintenance in the Plaice. (*P. platessa* L.). Part II . . . 877

A Statistical Study of Growth and Maintenance in the Plaice (*Pleuronectes platessa* L.) 949

FAOUZI, H.

Tube Formation in *Pomatoceros triqueter* L. 379

FORD, E.

Some Abnormal Fishes received at the Plymouth Laboratory . . . 53

Growth in Length during the Transition from Larva to Adolescent in the Pilchard and Sprat 977

Changes in Length during the Larval Life and Metamorphosis of the Freshwater Eel (*Anguilla vulgaris* Turt.). 987

GALL, D. C., AND ATKINS, W. R. G.

Apparatus for the Photo-Electric Measurement of Submarine Illumination assembled for the U.S.A. Research Ship, *Atlantis* 1017

GARDINER, A. C.

The Validity of Single Vertical Hauls of the International Net in the Study of the Distribution of the Plankton 449

HICKLING, C. F.

A New Type of Luminiscence in Fishes. III. The Gland in *Cœlorhynchus cœlorhynchus* Risso 853

HUXLEY, J. S., AND RICHARDS, O. W.

Relative Growth of the Abdomen and the Carapace of the Shore-Crab *Carcinus mœnas* 1001

IBAÑEZ, O. GOMEZ

On the Colorimetric Determination of pH in Sea-Water 483

JEPPS, M. W.

Note on a Marine Labyrinthula 833

LEBOUR, M. V.

The Larvæ of the Plymouth Galatheidæ. I. *Munida banffica*, *Galathea strigosa* and *Galathea dispersa* 175

The Larvæ of the Plymouth Galatheidæ. II. *Galathea squamifera* and *Galathea intermedia* 385

Clione limacina in Plymouth Waters 785

The Larval Stages of *Nassarius reticulatus* and *Nassarius incrassatus* . . 797

The Larval Stages of *Trivia europea* 819

LINDSAY, S. T., AND THOMPSON, H.

The Determination of Specific Characters for the Identification of Certain Ascidians 1

LLOYD, B.

Muds of the Clyde Sea Area. II. Bacterial Content 751

CONTENTS OF VOLUME XVII.

MACKINNON, D. L., AND RAY, H. N. PAGE

Notes on the Ciliate *Boveria stevensi* Issel from *Galeomma turtoni* Sowerby at Plymouth 577

An Amœba from the Intestine of an Ascidian at Plymouth 583

MOORE, H. B.

The Muds of the Clyde Sea Area. III. Chemical and Physical Conditions; Rate and Nature of Sedimentation; and Fauna 325

The Specific Identification of Fæcal Pellets 359

NICHOLLS, A. G.

Studies on *Ligia oceanica*. I. A. Habitat and Effect of Change of Environment on Respiration. B. Observations on Moulting and Breeding . . . 655

Studies on *Ligia oceanica*. Part II. The Processes of Feeding, Digestion and Absorption, with a Description of the Structure of the Foregut . . . 675

ORTON, J. H.

On the Effect of the Severe Winter of 1928–29 on the Oyster Drills with a Record of Five Years' Observations on Sea-Temperature on the Oyster-beds of the Blackwater Estuary 301

ORTON, J. H., AND AMIRTHALINGAM, C.

Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part II. On the Gonad of Egg-Spawning Individuals . . . 315

PAYNE, N. M.

Hydroid Pigments. I. General Discussion and Pigments of the Sertulariidæ 739

POOLE, H. H.

See ATKINS, W. R. G.

RAY, H. N.

See MACKINNON, D. L.

REGNART, H. C.

The Lower Limits of Perception of Electrical Currents by Fish . . . 415

RICHARDS, O. W.

See HUXLEY, J. S.

RUSSELL, F. S.

The Vertical Distribution of Marine Macroplankton. X. Notes on the Behaviour of Sagitta in the Plymouth Area 391

Notes on Cyanea Caught in the Ring-trawl in the Plymouth Area during the Years 1925 to 1930 573

The Vertical Distribution of Marine Macroplankton. XI. Further Observations on Diurnal Changes 767

SANDERSON, A. R.

The Cœlenterate Plankton of the Northumbrian Coast during the Year 1924 . 219

SEN, PURNENDU.

Preliminary Observations on the Early Stages of *Scatella stagnalis* Fal. (Ephydridæ, Diptera) 847

SEXTON, E. W., CLARK, A. R., AND SPOONER, G. M.

Some New Eye-Colour Changes in *Gammarus chevreuxi* Sexton. Part I . . 189

CONTENTS OF VOLUME XVII.

SPOONER, G. M. PAGE

Some Observations on Schooling in Fish 421

See also SEXTON, E. W.

STEPHEN, A. C.

Notes on the Biology of Certain Lamellibranchs on the Scottish Coast . . 277

STEVEN, G. A.

Rays and Skates of Devon and Cornwall. Methods of Rapid Identification on the Fishmarket 367

STANBURY, F. A.

The Effect of Light of Different Intensities, Reduced Selectively and Non-selectively, upon the Rate of Growth of *Nitzschia closterium* 633

THOMPSON, H.

See LINDSAY, S. T.

WATSON, H. G.

The Cœlenterate Plankton of the Northumbrian Coast during the Year 1925 . 233

ABSTRACTS OF MEMOIRS RECORDING WORK DONE AT THE PLYMOUTH LABORATORY.

Some Geochemical Applications of Measurements of Hydrogen Ion Concentration. By W. R. G. Atkins 1029

Observations on the Photo-electric Measurement of the Radiation from Mercury Vapour Lamps and from the Sun, and on the effects of such Radiation upon the Skin. By W. R. G. Atkins 1032

Some Experiments on the Accuracy obtainable with Gas-filled Photo-electric Cells. By W. R. G. Atkins 1032

The Penetration of Light through successive Layers of Tissue-paper. By W. R. G. Atkins 1033

The Treatment of Gapes in Chicken. By W. R. G. Atkins 1033

The Distribution of Pasture Plants in Relation to Soil Acidity and other Factors. By W. R. G. Atkins and E. W. Fenton 1030

The Photo-electric Recording of Daylight. By W. R. G. Atkins and H. H. Poole 1029

On the Photo-electric Measurement of Daylight. By W. R. G. Atkins and H. H. Poole 1030

Photo-electric Measurements of Illumination in Relation to Plant Distribution. Part 4. Changes in the Colour Composition of Daylight in the open and in shaded situations. By W. R. G. Atkins and H. H. Poole 1031

Photo-electric Measurements of Illumination in Relation to Plant Distribution. Part 3. Certain Spruce, Larch, Oak and Holm Oak Woods. By W. R. G. Atkins and F. A. Stanbury 1030

The Adductor Mechanism of Pecten. By L. E. Bayliss, E. Boyland, and A. D. Ritchie 1033

Studies in Tunicate Development. Part I. General Physiology of Simple Ascidians. By N. J. Berrill 1034

Studies in Tunicate Development. Part II. Abbreviation of Development in the Molgulidæ. By N. J. Berrill 1035

Regeneration in *Sabella pavonina* (Sav.) and other Sabellid Worms. By N. J. Berrill 1035

Untersuchungen zur Physiologie der Tonusmuskeln. By E. Bozler 1036

Notes on the Hydrogen Ion Concentration Excess Base and Carbon Dioxide Pressure of Marine Aquarium Waters. By E. M. Brown 1037

PAGE

The Action of Potassium Cyanide and Potassium Ferricyanide on certain Respiratory Pigments. By S. F. Cook 1037

The Anatomy and the Histology of Bud-Formation in the Serpulid *Filograna implexa*, together with some Cytological Observations on the Nuclei of the Neoblasts. By G. H. Faulkner 1037

A Spectrographic Analysis of Animal Tissues. By H. M. Fox and H. Ramage . 1038

Notes on Protodrilus. By E. S. Goodrich 1039

On the Morphology, Feeding Mechanisms, and Digestion of *Ensis siliqua* (Schumacher). By A. Graham 1039

On the Optimum Hydrogen Ion Concentration and Temperature of the Style Enzyme of *Pecten maximus*. By A. Graham 1040

On the Correlation of the Life-history of the Acephaline Gregarine, Gonospora, with the Sexual Cycle of its Host. II. *Gonospora* (*Kulpidorhynchus*) *arenicolæ*. By C. C. Hentschel 1041

Regeneration of the Spines in Sea-Urchins. By A. D. Hobson 1041

Carbohydrates of Crab Nerve. By E. G. Holmes 1041

Observations on Dicystid Gregarines from Marine Worms. By D. L. Mackinnon and H. N. Ray 1042

A New Protozoon, *Hyperidion thalassemæ* n. gen., n. sp., from the Intestine of *Thalassema neptuni* Gærtner. By D. L. Mackinnon and H. N. Ray . . 1042

The Feeding Mechanism, Formation of the Tube and Physiology of Digestion in *Sabella pavonina*. By E. A. T. Nicol 1042

Giant English Oysters. By J. H. Orton 1043

On the Oyster Drills in the Essex Estuaries. By J. H. Orton 1043

Oysters and Oyster Culture. By J. H. Orton 1044

The Adaptation of *Gunda ulvæ* to Salinity. I. The Environment. By C. F. A. Pantin 1045

The Adaptation of *Gunda ulvæ* to Salinity. II. The Water Exchange. By E. Weil and C. F. A. Pantin 1045

The Adaptation of *Gunda ulvæ* to Salinity. III. The Electrolyte Exchange. By C. F. A. Pantin 1046

Do Oceanic Plankton Animals lose themselves? By F. S. Russell . . . 1046

Vitamin Content of Marine Plankton. By F. S. Russell 1047

The Swimming of Cuttlefish. By F. S. Russell and G. A. Steven . . . 1047

Digestive Processes in Marine Invertebrates and Fishes. By C. M. Yonge . . 1047

The Significance of the Relationship between Corals and Zooxanthellæ. By C. M. Yonge 1048

The Crystalline Style of the Mollusca and a Carnivorous Habit cannot normally coexist. By C. M. Yonge 1048

Errata.

p. 741, line 31, for *Sargartia* read *Sagartia*.

p. 808, Explanation to Plate 1, Fig. 1, for 0·48 mm. read 4·8 mm.

The Determination of Specific Characters for the Identification of Certain Ascidians.

By

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and

Harold Thompson, M.A., D.Sc,

Fishery Board for Scotland, Aberdeen.

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With Plates 1–8 and 1 Figure in the Text.

CONTENTS.

		PAGE
	Introduction	1
1.	Comparative Study of the Forms recently included in Gen. *Ascidiella* .	3
2.	Variation in other Species of Fam. *Ascidiidæ*	20
3.	A suggested Key to the Boreal Species of Fam. *Ascidiidæ*. . .	29
4.	Synonymy	32
5.	Summary	33
6.	References	34
	Plates 1–8	36

Introduction.

In the course of a study involving the routine identification of Tunicata the need became apparent of an attempt being made to define more clearly than has been done the diagnostic characters of certain species. The examination of a widely-collected material demonstrates the fact that in certain species considerable variations in structure accompany changes of habitat (depth, latitude, temperature, type of substratum, etc.), while other species vary but slightly. Thus if reference be made to the most recent comprehensive work on British Tunicates (**2**) it is found that in some cases many species—all mere varieties of one definite species (e.g. *Ascidia mentula*)—have been described, while in others (e.g. *Ascidia obliqua*) the features of but one form are given, there being no confusion due to apparent modifications of that form. Hartmeyer (**6**) revised Alder and Hancock's list of species, uniting many closely related forms under specific names not always adhered to in his later work (**7**). In many cases his conclusions were inevitable and have been accepted by more recent workers on the phylum. In connection with certain other cases, however, a distinct tendency has developed to consider that he has subordinated under one name two or more forms sufficiently clearly demarcated to warrant some kind of separate recognition. Thus Ärnbâch

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(**15**) and Berrill (**3**) have *inter alia* devoted more special attention to the Family *Molgulidæ*, without, however, leaving the systematic position quite clear. It is a particular merit of the latter author's work that he redirects attention to the importance of developmental characters, concerning which a vast amount of accurate information seems yet to be lacking. At the same time, on practical grounds it is requisite that ontogenetic peculiarities should be correlated with recognisable differences of adult structure, since specific diagnosis must perforce most often be made upon preserved material. Definition of the range of variation of both developmental and adult characters is the desideratum.

Within the limits of the available facilities this paper deals with the type Family *Ascidiidæ* of the phylum, and gives the results of a semi-statistical study of material collected over a wide area (with northern and southern limits of Iceland and the English Channel respectively) wherein the nature of the habitat is sufficiently diversified to give rise to at least a fair amount of structural variation.

With two exceptions the species dealt with here were at various times kept in the live state either on board a research vessel or in the laboratory,* the object being to obtain for study the eggs, larvæ, and early fixed stages. This object was imperfectly attained. Thus species such as *A. mentula*, *virginea*, *conchilega*, and *mammillata*, which are not indigenous in the sea near the laboratory, and which had to be transported thither from a distance, gave rather meagre results. Even on the west Scottish coast, where they are common, and where they were kept on board ship, *A. mentula* and *A. conchilega* did not readily shed the reproductive elements. By contrast *A. aspersa* and *A. scabra* –particularly the latter—displayed enormous reproductive activity whether kept aboard ship or in the laboratory, in the neighbouring waters of which only the latter of these species is indigenous. The shedding of eggs could, in the case of *A. scabra*, usually be induced if necessary by the slight raising of the temperature of the water, but in no case did the other species respond to this stimulus. It is perhaps owing to its great fecundity that *A. scabra* is by far the commonest British Ascidian.

Two or more individuals of each species were kept in large aerated jars. No food was given except that contained naturally in the frequent changes of sea-water supplied. Under these conditions the smaller animals appeared to live quite healthily for months, but large individuals of *A. mentula*, *mammillata*, and *aspersa* could not be kept in condition for more than two or three weeks. No attempt at artificial fertilisation of eggs was made. The latter could be seen being intermittently expelled. In the case of any one individual the shedding of eggs in groups would proceed thus for 24–36 hours during spells of activity occurring every few weeks.

* Scottish Fishery Board, Aberdeen.

Development of the eggs as far as the larval stage was always easily obtained, but under the conditions the metamorphosing larva almost always died before reaching the fixed stage. Only in the case of *A. scabra* was the fixed stage showing protostigmata obtained. The specific evidence submitted below therefore relates mainly to the production of certain forms of eggs and larvæ by species whose body characters are defined.

Roule (**20**) suggested the establishment of 3 genera within what is now recognised as Fam. *Ascidiidæ*, and these have been accepted (with some reservation) by other authors, partly, we think, since the establishment of the genus Ascidiella segregated a set of troublesome and confusing forms, leaving the genera Ascidia and Phallusia to be devoted to easily distinguishable species. It is to the study of the constancy and the relative specific value of the developmental and anatomical characters of the confusing forms more recently recognised under Genus *Ascidiella* that this paper is chiefly devoted. Collateral work has, however, been done on a scantier material relating to the other species of the Family. For reasons to be given below we incline to the opinion that, in the light of the facts presented here, the retention of the Ascidiella forms in a separate genus is not warranted.

1. Comparative Study of the Forms recently included in Genus Ascidiella (Roule, 1883, modified).

As treated by Hartmeyer (**7**) Gen. *Ascidiella* contains only one species —*A. aspersa* (Müll. 1776). It was that author's considered opinion that the three forms recognised by Herdman (**12**) are mere varieties of this species. The latter author did indeed call attention, as did Roule, to the existence of transitional forms apparently linking up one with another of his types. The first and largest type was named *Ascidiella aspersa ;* the two others—*A. virginea* and *A. scabra*—were much smaller and could be inter-distinguished by their habit, the former being erect, the latter recumbent and attached by the greater length of its side.

Roule's definition of Gen. *Ascidiella* brought in three Ascidian species (*A. virginea*, *obliqua*, *prunum*) which have since been excluded by the alteration of that definition to the following :—

a. Nerve ganglion almost directly above the dorsal tubercle.

b. The branchial papillæ are reduced.

It is chiefly on the constancy of character *b* that Gen. *Ascidiella* is based. All that is, however, implied by the term reduction is that free papillæ, as found in other species (Plate 7, Fig. 3), do not occur, although in certain portions of the branchial sac—e.g. near the mouth—a rudimentary state may persist (Plate 7, Fig. 2). In the latter case irregular

bosses arise from the transverse vessels and either remain isolated or become more or less linked up with each other by the outgrowth of longitudinal spurs. In cases where these bosses are numerous they might be mistaken for papillæ. Normally, however, they are completely linked up by longitudinal vessels (Plate 7, Fig. 1) and are seen only as thickened portions of these bars. Instead of growing out into true free papillæ they have the appearance of being repressed back on to the transverse vessels by means of their association with a transverse papillary membrane which links them together laterally. The experienced worker has no difficulty in recognising Gen. *Ascidiella* from this peculiarity of sac structure.

The point to be investigated here is the number of species capable of clear distinction within this genus. Can the reduction from Herdman's three forms to Hartmeyer's one be justified ? The latter author certainly has not submitted a thorough analysis of the forms. Berrill claims that at least two, and possibly three, species are to be distinguished. We may recapitulate the points in which he finds form *aspersa* to differ from form *scabra*.

A. aspersa.—Attached basally ; attains maximum size of 12–13 cm. ; any mantle pigment present is localised near the siphons ; has the renal bodies extensively distributed over the mantle ; has floating egg, with very large outer follicle cells.

A. scabra.—Attached by side ; size restricted to 4 cm. ; has red pigment spread usually over most of the mantle and has the atrial siphon relatively nearer the branchial than is the case in *A. aspersa ;* the renal bodies are less extensively distributed ; the egg is non-floating, with small outer follicle cells and the chorion raised further from the ovum surface than is the case in the egg of *aspersa*.

The third possible form (Herdman's *A. virginea*) is said to differ from form *scabra*, which it most resembles, in possessing a deep, diffusely spread brown pigment, and in forming eggs in which the perivitelline space is narrower.

At the outset of this study an attempt was made to keep forms resembling *scabra* and *virginea* separate, but no justification could be found for so doing. Their eggs and larvæ appear to be identical respectively, and in outward appearance the two extremes of form are linked up by a range of intermediate forms. In addition there is no consistent anatomical difference between them, although in certain habitats the erect, in others the recumbent form may be that more commonly found. The characters of the two forms and their intermediaries are therefore combined here and are submitted under the name *scabra*, thus being contrasted with those of form *aspersa*.

We have had the opportunity of examining the material from over

200 collections of form *scabra* and from 11 collections of form *aspersa*, which is obviously a comparatively rare species in British waters. While dissections were made only on the following specimens selected from this wider material, it is to be emphasised that rougher examination of the remainder served to confirm the conclusions arrived at.

A. aspersa.—22 specimens, length 7–76 mm., taken from shallow water, Plymouth ; 11 spec., 30–72 mm., 41 metres, Loch Fyne ; 2 spec., 37–50 mm., 40 metres, Loch Duich ; 1 spec., 18 mm., 10 metres, Lochmaddy (North Uist) ; 1 spec., 42 mm., between tide-marks, Olna Firth, Shetland ; 25 spec., 8–31 mm., 7–12 metres, Loch Indail, Islay ; 2 spec., 37–56 mm., 7–17 metres, Ettrick Bay.

A. scabra.—13 spec., 8–29 mm., Eddystone Grounds ; 11 spec., 28–35 mm., 11–20 metres, Luce Bay ; 30 spec., 12–43 mm., 48 metres, Firth of Clyde ; 7 spec., 27–35 mm., 41–138 metres, Loch Fyne ; 5 spec., 20–29 mm., 16–28 metres, Islay ; 15 spec., 22–36 mm., 80 metres, between Skye and Mull ; 30 spec., 15–38 mm., 103 metres, Hoy, Orkney ; 30 spec., 6–34 mm., 123 metres, Scalloway, Shetland ; 38 spec., 16–31 mm., 142 metres, E. of Balta, Shetland ; 15 spec., 15–37 mm., 107 metres, Northern North Sea ; 43 spec., 6–47 mm., 62 metres, Northern Moray Firth ; 10 spec., 15–41 mm., 82 metres, 50 miles E.S.E. of Aberdeen ; 18 spec., 9–39 mm., 15 metres, Kirkcaldy Bay ; 30 spec., 24–73 mm., 36 metres, Dogger Bank (north edge); 30 spec., 19–53 mm., 42 metres, Dogger Bank (Tail End).

The following are the results of the investigations of the characteristic features of these two forms :—

(A) *Geographical and Bathymetrical Distribution.*—If two otherwise closely allied forms have precisely the same distribution the case for considering them the same species is strengthened. Owing to confusion in nomenclature it is not always clear to which form authors have referred, but it can be said that *A. aspersa* is in general found in much shallower water than is *A. scabra*. The former is commonest in harbours, river estuaries, etc., and can succeed in brackish water ; it is found coastally from the Mediterranean to the latitude of Shetland, but as far as is known it is absent from the east British coast (except from the Thames estuary). Although it occurs on the south Norwegian coast it is doubtful if it extends much distance northwards along the west coast. The records from Ettrick Bay, Islay, Loch Duich, and Olna Firth are new. North of the English Channel this species is rare. *A. scabra*, however, is taken in great numbers at all points where its congener occurs ; it favours deeper water (5–300 metres as compared with

0–50 metres), and is the commonest Ascidian of the North Sea, where *A. aspersa* is lacking. It also extends further north than that species, being found at Faroe and Trondhjem. There is therefore far from complete coincidence between the distribution of the two forms. At the same time they often occur in close association, and were they similar in almost all other respects might be considered as shallow- and deep-water forms of the same species.

(B) *Size.*—In the English Channel where it is fairly common *A. aspersa* can, according to Berrill, attain 13 cm. in size. The average size is, however, probably no more than 6–7 cm. Scottish specimens tend to be smaller even than this. *Scabra* rarely exceeds 2–3 cm. in mean size, and 4–5 cm. in maximum size. On the Dogger Bank, however, a maximum size of 7½ cm., if not more, is reached. Conversely the maximum size in areas in which the recumbent form predominates is seldom more than 3½ cm. (All these measurements, with the exception of Berrill's, refer to the antero-posterior dimension, omitting the length of the siphons, which varies with the extent of their contraction.) *Aspersa* is therefore, on the average, at least twice as large as *scabra*.

(C) *Attachment to Substratum.* There is by no means uniformity of degree of fixation in either *aspersa* or *scabra* (or, for that matter in most other species). Thus although *A. aspersa* has hitherto been regarded as a basally attached animal, we have obtained in Loch Indail, Islay, a group of specimens (Plate 5, Fig. 2) attached by nearly the whole side. These occurred in the shallow water of a bay exposed to the scouring action of south-westerly swells, and erect, slightly attached individuals would in such a position encounter severe buffeting. The fixation was to a large Cyprina valve which was firmly silted down into the sand. These animals were not dissected since it was desirable to retain them as type specimens, but they resembled *A. aspersa* in size, nature of test, position of apertures, and type of egg. The occurrence of adult individuals so fully attached is doubtless very exceptional. In the Plymouth material 23 individuals were fixed basally and one by half of its side. Twenty of the Loch Fyne animals were attached basally, and two by half the side. It is noteworthy that in the Plymouth material of *Ascidia virginea*, a species hitherto thought always to be erect, a parallel case was encountered (Plate 4, Fig. 6) where an individual was wholly attached by its side to the valve of a Scallop.

Turning now to *A. scabra*, we find that there is no justification for the establishment of two species (*scabra* and *virginea*) on the score that one is attached by the whole or the greater part of the side, the other basally. All Dogger Bank specimens were found to be basally fixed, either to a Lamellibranch valve (Plate 4, Fig. 4) or by means of a conical root-like prolongation of the test substance into the sand. All of the

Firth of Clyde, Orkney, and Islay individuals were also attached by the bases. In the other areas (Plymouth excepted) practically all were so fixed, but a greater or less tendency was displayed for certain individuals to have more extensive attachment (Plate 4, Fig. 1). Only of the Plymouth material could it be said that the predominant tendency was towards complete attachment.

Attachment.	Plymouth.	N. North Sea.	Luce Bay.	Scalloway.	Skye–Mull.	Kirkwall.	Loch Fyne.	Moray Firth.	Balta.	E.S.E. Aberdeen.
Basal	3	10	29	26	12	16	6	39	37	64
One-third of side . .	2	–	3	–	1	–	–	1	–	3
Half of side . . .	7	1	2	2	1	–	1	3	1	6
Two-thirds of side .	7	1	–	–	–	–	–	–	–	–
Three-fourths of side .	3	2	2	1	1	2	–	–	–	–
Whole side . . .	–	1	1	1	–	–	–	–	–	–

It is probable that the recumbent form tends most frequently to be found where stones, shell-valves, seaweed-fronds, etc., occur to afford the basis of attachment, and where, in addition, a large average size is not attained. Thus in the deep water of the northern North Sea, in very soft mud, it is natural to find more than normal use being made of hard objects for means of attachment. Any attempt, however, to establish constant points of anatomical difference as between flat and erect forms has been unsuccessful. On the other hand, as will be shown below, constant points of resemblance are capable of easy demonstration.

(D) *Colour.*—*A. aspersa* has been stated to differ from *A. scabra* in being practically colourless, whereas in adult individuals of the latter the mantle is usually more or less reddish-brown. This distinction can be said to hold in most cases, but it frequently breaks down. Our material has provided no pigmented forms of *aspersa*, but Alder and Hancock have illustrated their description of *Ascidia Normani* (=*A. aspersa*) by means of a figure showing a brilliantly carmine-pigmented mantle, such as is often found in *A. scabra*. This does not agree with their description of the mantle as being of a pale buff flesh-colour or almost colourless. We conclude that the specimen figured is one of the exceptional pigmented forms, called by these authors *A. Normani* (var. *resplendens*).

On the other hand, almost or quite colourless forms of *A. scabra* are frequently found. For example, the large Dogger Bank individuals have at most the pale buff colour referred to above, while the beautifully transparent specimens from the inner Moray Firth (Plate 4, Fig. 3) are virtually free from pigment. Even the flat forms have their colourless variety (*A. scabra* var. *albida* of A. and H.).

The above distinction therefore applies only to the majority of cases and is not strictly specific.

(E) *Nature of Test.*—Here we deal with a character which falls just short of being absolutely specific. In *A. aspersa*, as the name signifies, the test of the young animals is more or less covered with fine papillæ. In cases where no pigment develops (e.g. in the west Highland sea lochs) the specimens present, at size 2–4 cm., an attractive frosted appearance. In larger animals, however, the test becomes more or less coarsely pustulose (Plate 5, Fig. 1) and is cartilaginous in consistency, rather than gelatinous or coriaceous as in *A. scabra*, in which it is usually smooth or at most wrinkled, with occasionally a few papillæ round the siphons. The test in the neighbourhood of the siphons may indeed be strongly echinated (as in A. and H's *A. scabra* var. *lævis*), but it is possible that the var. *echinata* (Lamlash Bay, Arran) of these authors is a young stage of *A. aspersa*, since the papillæ extended over the test generally.

(F) *Position of the Apertures.*—It has been held to be a distinguishing feature of *A. aspersa* that the atrial aperture is separated from the branchial by a distance equivalent to rather less than one-half of the body-length ; whereas in *A. scabra* the distance is only one-quarter (and rather less than one-quarter in the recumbent forms). If we express the distance between the mid-points of the siphons as a fraction of the body-length we find that out of 65 specimens of *aspersa* 32 gave one-third, 7 rather less than one-third, and 26 rather more than one-third. That is, the mean distance is in these cases rather greater than one-third, and the range of variation is from about one-fourth to just less than one-half.

In the case of *A. scabra*, out of 446 specimens 71 gave a ratio of one-fifth, 311 of one-fourth, 58 of one-third, and 6 of one-half. The tendency towards the larger interval existed in areas in which the recumbent form was most numerous. The mean interval between the apertures was therefore about one-fourth of the body-length, and the variation on either side of that ratio was approximately balanced as between one-third and one-fifth.

Where it is desired to distinguish the species without dissection the distinction (between the mean ratios of one-third and one-fourth respectively) is of accessory value, but as has been shown some overlapping of the respective ratios occurs.

A note may be made here of the very rare occurrence of the persistence of an early post-larval character in *A. scabra*. From the Doghole, off Aberdeen, a specimen was taken with two atrial siphons (Plate 8). Herdman (**10**) records a similar case from Llandudno.

(G) *Numbers of Branchial Stigmata per Mesh.*—A mesh of the branchial sac may be defined as the rectangular area enclosed by the intersection of two adjacent longitudinal with two adjacent transverse vessels. Herdman

(12) gave it as a distinction of *A. aspersa* that from 4–6 stigmata are formed in each mesh, as compared with from 7–12 in the recumbent form of *A. scabra*. Now were this the case there would be, in the number of stigmata, a specific point of difference between the two species.

The main features of a count of the stigmata in 29 specimens of *A. aspersa* and 316 specimens of *A. scabra* are shown in the following table, in which size 5 includes specimens ranging in size from 3–7 mm., size 10 those ranging from 8–12 mm., and so on. If it be remembered that *A. aspersa* is, age for age, much larger than *A. scabra*, it is probably the best interpretation of the results to deduce that, stage for stage, though not of course size for size, both forms have approximately the same number of stigmata per mesh, and that this number increases with age to a rather definite maximum average number (6–8). Now this maximum is attained by *A. scabra* at a smaller size than by *A. aspersa* (witness the attainment of the maximum range—4 to 9 at size 20 mm. in the case of the former, and, in that of the latter, the maximum of 6 to 8 at size 40 mm.).

No. of Stigmata per Mesh.

Size (mm.).	*A. aspersa*.			*A. scabra*.		
	No. of Specimens.	Range.	Mean.	Mean.	Range.	No. of Specimens.
5	–	–	–	3·3	3–4	2
10	1	2	2·0	4·0	3–6	7
15	6	3–4	3·7	4·7	3–7	16
20	2	4–5	4·3	5·2	4–9	57
25	4	4	4·0	6·0	4–9	68
30	3	5	5·0	6·5	4–9	85
35	–	–	–	6·9	4–9	44
40	13	6–8	7·0	7·3	5–9	18
45				7·3	6–8	4
50–75				6·8	6–8	15

The similarity of the figures for both species at sizes 40–75 is probably due to the fact that in the case of *A. scabra* we are at this stage dealing only with the fast-growing Dogger Bank specimens which, age for age, must approach in size the specimens of *A. aspersa* which have been examined. Neither species can be said to increase its average number of stigmata after a size of about 40 mm. is attained. Unfortunately the table does not show features brought about by differences in growth rate in one area as compared with another. It is informative, for example, to take separately the results for those areas where a small maximum size (35 mm.) is attained by *A. scabra*. In Luce Bay and Loch Fyne individuals 7 stigmata per mesh are found at a size of only 15 mm., whereas from all areas the average is below 5. Again, at size 20 mm., the average number is nearly $7\frac{1}{2}$ as against an all-over average of just over 5. Not only is full stigmatic development reached in these areas at a relatively small size, but the high maximum number of stigmata of 9 is formed. This number is equalled elsewhere only in the northern Moray Firth, but

is attained at the relatively larger size of 30 mm. In the material examined the highest individual number of stigmata found was 10 (the figure 9 in the upper limits of the ranges of the above table indicating ranges of 8–10). For all the areas other than Luce Bay, Loch Fyne, and the Moray Firth the maximum number of stigmata found was slightly less — 8 or 9—and was attained in each area at a size roughly proportional to the ultimate maximum size attained in that area. The effect of combining the Luce Bay, etc., figures with those from other areas has therefore been slightly to raise the mean number of stigmata attained at the lesser sizes, and may for comparative purposes be ignored since the numbers of individuals examined from the latter areas were by far the greater.

In the case of *A. scabra* we have probably dealt with collections including some specimens which had reached the maximum size attainable by this species. In that of *A. aspersa*, however, the largest animal was 76 mm. in size, so that individuals up to fully half as large again remain to be examined. Whether the latter will exhibit a larger mean number of stigmata than 9 (the highest encountered in our material) seems doubtful. In justifying this opinion we may add information not revealed in the above table, which is a summary. At mean sizes of 40, 45–75 mm. the following were the ranges in the number of stigmata in the individual cases (in ascending order of size) : 7–8, 5–7, 6–8, 7–9, 5–9, 5–9, 7–9, 7–9. Thus the range as well as the mean number of stigmata remained practically stationary although the size of the animal increased to almost double.

The general inference from these results is that size for size (up to a size of about 35 mm.) *A. aspersa* has, on the average, from 1–2 fewer stigmata per mesh than has *A. scabra*, and that in certain areas (e.g. Loch Fyne) where both species occur the difference may be rather more (3 or at most 4). In the general routine of distinguishing the species, however, little aid will be obtained from counting the stigmata.

(H and J) *Number of Tentacles and of Internal Longitudinal Bars.*— These two characters are taken together since a relationship of specific value can be shown to exist between them. Little has hitherto been done with regard to enumerating the number of bars, and estimates of the number of tentacles have varied considerably, part of the variation being due to inaccurate observation, to which Herdman (**12**) calls attention. The method here adopted was to discard individuals which had been indifferently preserved (in the sense that the branchial aperture was closed by the sphincter muscles and the dissected-out tentacular collar so contracted that the tentacles could not be counted with at least 95% accuracy). The tentacles stain very well with very dilute hæmatoxylin so that even the most rudimentary ones can be easily seen and enumeration be made by turning each filament over with the needle under the

binocular microscope. In certain cases (small individuals of *A. scabra*) it was necessary in order to secure a non-convolute piece of tissue to cut the collar into about 4 equal portions, and to make the count in each of the latter separately. Repeat counts should correspond to within the degree of accuracy stated above. In well-preserved material discards need seldom be made. The longitudinal bars were enumerated in a transverse direction in the right half of the branchial sac, immediately above the mouth.

The results of the counts made on the two species are plotted against size (Fig. 1). In *A. aspersa the number of bars always outnumbers that of the tentacles* (B. and T., Fig. 1). This, at least, is the case in specimens of 10 mm. or more in size. Individuals from Islay and of size less than 10 mm. showed a reversal of this relationship. (A series of size 5–9 mm. gave an average of 31 tentacles as against 28 bars.) Variation in locality has some effect in causing variation in the number of tentacles, since, size for size, Islay specimens were found to have formed more tentacles than those from Plymouth. The results from the Islay form are not included in Fig. 1, but in animals ranging in size from 10–20 mm. there occurred from 29–34 tentacles (av. 31–32), while at the higher size of 20–30 mm. from 28–43 tentacles (av. 34–35) were found. Thus the Islay form agrees in this character with the forms from Lochmaddy and Shetland (represented in Fig. 1), the number of tentacles being greater than that developed in the forms from Plymouth, Loch Fyne, and Ettrick Bay. (One specimen of size 56 mm. from the last-named locality had 36 tentacles and 50 bars, ostensibly classing it with the Loch Fyne type.)

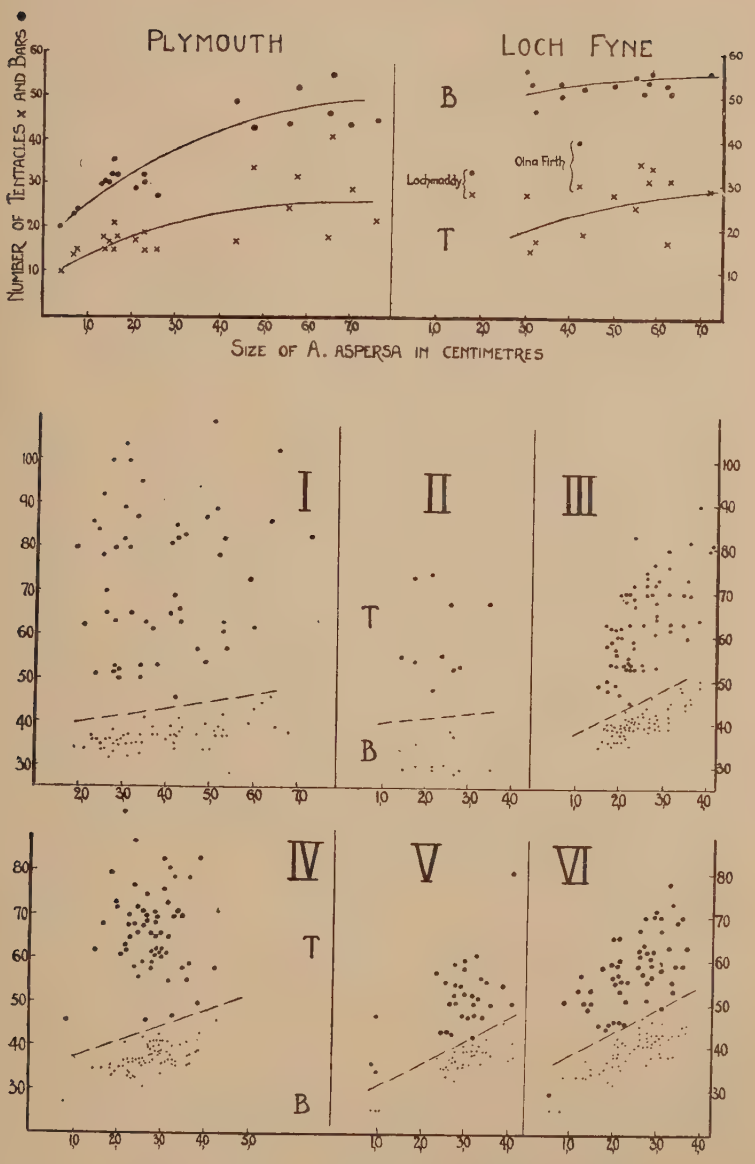
With regard to the number of bars, it was found that Loch Fyne and Ettrick Bay specimens had a slightly greater number than those of Plymouth, and a much greater number than those of Islay, Lochmaddy, and Shetland. The figures for the Islay forms may be given here since they are not represented in Fig. 1. From sizes 10–20 mm. there were from 32–39 bars (av. 36–37), and from sizes 20–30 mm. from 36–42 bars (av. 40). In every case the number of bars exceeded that of the tentacles but not by so large a margin as in the other areas, which, it may be noted, agree in being less directly exposed to oceanic conditions. In another sheltered area—Loch Duich—one specimen of 37 mm. was found with 54 bars (the tentacles could not be counted), and would apparently fall into the Loch Fyne class.

In the case of *A. scabra* (I–VI in Fig. 1) *the number of tentacles is always greater than that of the bars*—thus reversing the relationship found to hold for *A. aspersa*. For reasons of economy of space the results for the different areas have been grouped where similar. Thus I represents those for the North edge of the Dogger Bank as well as those for the Tail End of the Bank ; II those for Luce Bay alone ; III those for the area E.S.E. of

Aberdeen, as well as for Orkney and Shetland (Balta); IV those for the Firths of Clyde and Forth, for Islay, and for the area between Skye and Mull; V those for the Moray Firth alone; and VI those for the northern North Sea, for Scalloway (Shetland), for Loch Fyne and for Plymouth. The large dots represent the number of tentacles (T), the small dots the number of bars (B), both plotted against size of animal. As was the case in connection with *A. aspersa* both the number of tentacles and of bars appear to increase with size of individual—this as a general rule but, as was the case in the Islay, etc., forms of that species, the increase in number of tentacles is at most slight in I, II, and IV (in II also there is no obvious increase in the number of bars). The apparently fortuitous way in which areas (in some cases widely separated) gave sufficiently similar results to admit of the grouping of the latter in Figure 1 admits of no obvious explanation. Neighbouring areas, in which similarity of type might be expected to occur, as often as not exhibited dissimilarity of type. For example, the specimens from the two Dogger Bank areas, although agreeing in the size-number of bars relationship, disagreed to some extent in their respective number of tentacles, fewer being developed in those from the north edge. Again, in some areas the progressive increase (with size) in the number of bars or tentacles is much more rapid than in others. The fact of value to this investigation is, however, that a line can in every case be drawn to effect separation of the points representing the numbers of bars and tentacles respectively. *The determination of the relative number of bars and tentacles therefore affords information of diagnostic value with regard to the two species.* As these numbers appear usually to vary with size of individual no specific figures can be defined. The number of bars is much the same in both species (varying from 20–60 in *A. aspersa* and from 25–50 in *A. scabra*); but the number of tentacles varies from 10–40 in the former species and from 30–110 in the latter.

A second distinction lies in the fact that, owing to their comparatively great numbers, the tentacles are in the latter species very much crowded at the base, whereas those of *A. aspersa* are set at intervals and appear to be slightly flattened to a broader base than those of *A. scabra* (Plate 6, Figs. 27, 28).

(K) *The Shape of the Dorsal Tubercle* (Plate 6).—The shape of the opening of the duct from the neural gland affords a further means of distinguishing *A. aspersa* from *A. scabra*. In the former species this opening is transversely oval or elliptical (broader than high); in the latter it is practically always higher than broad. In very small specimens of both species the opening is in the form of a more or less crescentic or incomplete loop (Figs. 1, 15, 16), although in *A. aspersa* the typically broadly-oval contour is often attained even in very young animals. Size



(Explanation of I-VI in Text)

FIG. 1.

for size of individual, the tubercle of this species is larger than that of *A. scabra*, and examination of 60 specimens showed :—

a. 46 cases with transversely oval tubercle with both horns incurved (Figs. 2, 3).
b. 4 cases with transversely oval tubercle with both horns turned right (Fig. 5).
c. 7 cases with cordate tubercle (about equally as broad as high), and both horns incurved (Fig. 4).
d. 3 cases with cordate tubercle, with the right horn only incoiled.

In *A. scabra* great variation of the fundamentally U- or longitudinally-elliptical form occurs. Of 310 specimens :—

a. 75 had tubercle simple U- or staple-shaped (Figs. 11, 18, 22, 25).
b. 56 had tubercle U- or flask-shaped with both horns outrolled (Figs. 7, 23, 24).
c. 53 had tubercle U-shaped with both horns turned right (Figs. 10, 17).
d. 38 had tubercle U-shaped with only the right horn outrolled (Fig. 14).
e. 25 had tubercle broadly U-shaped with prolonged horns (Figs. 12, 20, 21).
f. 23 had tubercle U-shaped with left horn inrolled (Figs. 6, 13).
g. 14 had tubercle U-shaped with right horn inrolled (Fig. 19).
h. 13 had tubercle U-shaped with both horns inrolled (Fig. 8).
j. 9 had tubercle almost cordate in shape (Fig. 4).
k. 4 had tubercle U-shaped with left horn outrolled (Fig. 9).

Thus only in a relatively insignificant number of specimens (j) was the cordate type of tubercle to be found, and to this extent slight confusion with two of the minority forms (c and d) found in *A. aspersa* is possible, although in the latter species the size of tubercle is greater. In *A. scabra* the U- or modified U-shaped tubercle is clearly predominant, and in each area there is a particular tendency towards some two or three of the forms. The most striking departure from the fundamental shape was exhibited (e) by many of the Dogger Bank specimens, in which the tubercle was larger than usual and had the horns elongated much as is the case in another species, *A. obliqua*.

(L) *Distribution of the Renal Bodies.*—In both of the species under comparison these bodies are large (as seen in Plate 8, Fig. 2) and, besides being distributed over the mantle on the left side of the gut, extend also *well beyond* that region, whereas in other species of the Family *Ascidiidæ* they are usually said to be confined to the visceral region. This distinction between *A. aspersa*+*scabra* and the other species is probably specific if the phrase " well beyond " be used. (In *A. prunum* we have occasionally

found the renal cells to extend *slightly* beyond the region of the viscera, and Herdman (**12**) has found this to be the case also in *A. conchilega*). Berrill has claimed it as a distinction between *A. aspersa* and *A. scabra* that the renal cells are more widely dispersed in the mantle of the former. In our material there has been little to choose in this respect between the two species. At times the cells are difficult to detect owing to their transparency, and the extent of their distribution might then be overlooked. This was the case particularly in the Loch Fyne material of both species. In the Plymouth material, on the other hand, the cells were particularly obvious. The degree of clearness with which the renal cells are seen probably depends upon the amount and colour of the concretions they contain.

(M) *Size and Nature of the Eggs and Larvæ* :—

a. *The Eggs.*—Except in the case of two species the eggs of the members of the Family *Ascidiidæ* were obtained in the aquarium, and are depicted in Plate 1. In the cases of *A. obliqua* and *A. prunum*, however, living animals could not be obtained, and the size of the living egg was estimated from that of the ova in the terminal portion of the oviduct. (It was computed that the mean diameter of the living egg, one hour or so after expulsion, is on the average half as large again as that of the vitellus in the oviduct, most of the increase of size being due to the further withdrawal of the chorion from the vitelline surface.) Figures 7 and 9 are therefore included as showing, for comparative purposes, the probable sizes of the chorion in *A. prunum* and *A. obliqua* respectively.

It is important that the diameter of the recently expelled egg be that which is used for purposes of comparison, since some increase in diameter usually accompanies the development of the larva in the egg (Plate 2, Figs. 2, 3). The mean measurement of a number of eggs should, where possible, be used as a criterion, since at any one shedding period the eggs expelled are not of exactly the same sizes. Some difference in size may also be found if eggs from different regions, or eggs shed at different times of the year, are compared. It is of interest to note, however, that animals sent to Aberdeen from Plymouth, and kept in water from the sea off Aberdeen, yielded eggs differing in no way from those obtained from the same species in English Channel water. Slight differences of temperatures and salinity have therefore little or no effect on the structure of the egg. Certain species (*A. scabra, Dendrodoa grossularia, Ciona intestinalis*) which were kept in the aquarium over the winter season were found to yield eggs and larvæ even in March, although reproductive activity was in winter on a much reduced scale as compared with that in summer and autumn, when, incidentally, the largest eggs appear to be liberated.

The egg of *A. aspersa* differs from those of the other species of Family *Ascidiidæ* in possessing, at the time of liberation, an investing layer of very large follicle cells (Plate 2, Fig. 1). There are from 28–32 of these seen in optical section. The chorion is not at this stage obvious, whereas it can be seen surrounding the ovum even in the oviduct of *A. scabra*. In the newly-liberated egg of the latter species the outer follicle cells are much smaller, resembling those of the eggs of the other species so far examined. From 70–80 of them are seen in optical section and they form a layer over the chorion, which is quite clearly seen. The difference in the nature of the follicle cells can even be seen in groups of eggs dissected out from the terminal portion of the oviduct of each species (Plate 1, Figs. 10, 11). The difference in the size of the follicle cells, as Berrill states, is specific. Otherwise the eggs of these two species appear to agree in regard to diameter of vitellus and the nature of the cells which come to lie upon the surface of the latter.

Some time after the eggs of *A. aspersa* are set free the follicle cells separate to a greater distance from the ovum surface and are clearly seen to surround the chorion (Plate 2, Fig. 2). The result is that instead of being compressed owing to contiguity so as to present, in optical section, the appearance of a hexagonal network (Plate 2, Fig. 1), these large cells come to lie more or less free of each other (Fig. 2) so that they appear to be circular in optical section. Figure 1 represents an average size of egg of ·22–·24 mm. and Figure 2 of from ·28–·31 mm. The latter size persists unchanged for at least several hours, and may be taken as typical. The fertilised and developing egg (Plate 2, Fig. 3) may, however, be up to ·36 mm. in diameter. It is interesting to note that the average diameter of eggs obtained from Plymouth and West Scottish specimens of *A. aspersa* was found to be identical (from ·27–·32 mm.). As Berrill states the egg floats —in fact most of the eggs cluster together subjacently to the surface film of the water, and none is found further than one inch from the surface. This is not, however, the only species of Ascidian forming eggs displaying this character. Specimens of *Corella parallelogramma* taken in the Firth of Clyde and kept under observation on board ship yielded within a few hours similar but slightly smaller eggs (Plate 1, Fig. 8). These had a diameter of about ·18 mm. at the time of liberation, and of from ·24–·26 mm. one hour later. The eggs of *A. scabra*, however, do not rise to the water surface, remaining at or near the bottom of a jar containing them. At liberation they have the diameter and form which is maintained for many hours afterwards (Plate 1, Fig. 5 ; Plate 2, Fig. 4). It cannot be said that the chorion diameter differs materially from that of the eggs of *A. aspersa*, but this statement is made with reservation, since we have found that variation apparently arises if observations be

continued from season to season and upon individuals from different localities. The following variation in diameter has been found :—

Off Aberdeen, June. Size ·32–·36 mm. *Average* ·33 mm.
Off Aberdeen, Oct. Size ·27–·31 mm. *Average* ·29 mm.
Off Peterhead, June. Size ·34–·37 mm. *Average* ·345 mm.
Off Plymouth, June. Size ·30–·33 mm. *Average* ·32 mm.
Off Skye, August. Size ·28–·30 mm. *Average* ·29 mm.
Off N. Scottish Coast, Sept. Size ·29–·30 mm. *Average* ·295 mm.
Firth of Clyde, Aug. Size ·31–·32 mm. *Average* ·315 mm.

As Berrill figures an egg of only *ca.* 0·25 mm. diameter from the Swedish coast and one of *ca.* 0·40 mm. from the English Channel, it appears that even greater variation is possible. We have not, however, found any distinction in this character between erect and recumbent forms from the same area.

The conclusion is that *A. aspersa* may be distinguished from *A. scabra* and from all other species of Family *Ascidiidæ* by the nature of the outer follicle cells of the egg, and that this distinction may be effected even by dissection of the oviduct of the preserved mature animal. (In our material Scottish specimens of 29 and 31 mm. in length were found to be mature, while those in the region of size 24 mm. were immature. Berrill gives the size at first maturity as being not less than 40 mm. in the case of English Channel specimens.)

At the lower limit of its size-range the egg of *A. scabra* is not clearly to be differentiated from the eggs of *A. mentula* and *A. mammillata*. The two later species along with *A. conchilega* form relatively small eggs, viz :—

A. conchilega (Plate 1, Fig. 3). Diameter of egg *ca.* 0·22 mm.
A. mammillata (Plate 1, Fig. 4). Diameter of egg *ca.* 0·23–0·25 mm.
A. mentula (Plate 1, Fig. 1). Diameter of egg *ca.* 0·24–0·27 mm.

Again at the middle or upper portion of its size-range the egg of *A. scabra* might be confused with those of

A. prunum (Plate 1, Fig. 7). Diameter *ca.* 0·31–0·32 mm. (estimated), and

A. virginea (Plate 1, Fig. 2). Diameter *ca.* 0·34–0·35 mm.

All these species form eggs with relatively small outer follicle cells. The largest egg is evidently that yielded by *A. obliqua*, and, judging from the size of the ovum in the duct, must reach ·50 mm. in diameter (Plate 1, Fig. 9). In this case also the outer follicle cells are small, but the size alone would apparently be a feature of diagnostic value.

In view of the new scheme for the classification of Family *Ascidiidæ* suggested below, notice may be directed here to the fact that the eggs of

A. mentula, *conchilega*, and *mammillata* are very alike, and are the smallest formed in the Family. Examination of further material is, however, desirable to determine the precise limits of variation in the diameter of the eggs of these species, none of which, either on board ship or in the aquarium, freely shed their ova.

In passing it may be mentioned that, as is shown in Plate 2 (Figs, 5, 6), the rupture of the chorion by the larva appears, in this Family, to occur at a point opposite the anterior portion of the tail of the larva, this portion serving as a fulcrum for the stressing convulsive movements of trunk and tail.

b. *The larvæ.*—From *A. aspersa* and *A. scabra* from various regions larvæ were easily and repeatedly obtained. Only a few were, however, secured from *A. mentula* and *A. mammillata*, and none from *A. conchilega* and *A. virginea*. Two or more adult individuals of a species were maintained in the same jar. No attempt was made to obtain larvæ by means of artificial fertilisation. In Plate 3 there are represented the larvæ of the first four of the above-mentioned species. Save in Figures 1 and 3a the hyaline ectodermic mantle is omitted. The view of the subjects is such that only in Figure 3a are all three anterior adhesive papillæ shown, while Figures 2 and 5 show larvæ not long after the escape of the latter from the egg, so that the papillæ are more or less repressed, as they appear in the egg (Plate 2, Figs. 3, 5, 6). It is however obvious that the papillæ are, in all four species, of a short, truncated cone-like form. Allowing for the fact that in Figures 3, 4, and 5 the larvæ are viewed from the right side, while in Figures 1, 2, and 3a they are seen from the left (in Fig. 1 rather obliquely), it seems that there is no essential difference in any one species, as compared with another, in the relative positions of otocyst and eye in the cerebral vesicle. In some of the figures of Plates 2 and 3, however, different stages of notochordal development are shown. In the case of *A. scabra* (Figs. 3 and 3a of Plate 3, and Fig. 5 of Plate 2) the sheath of the notochord surrounds a hyaline core, and this is the only stage which we have observed in the larvæ of this species. In *A. aspersa* (Plate 2, Fig. 3), however, both in the developing larva in the egg and in the newly-hatched larva, the hyaline substance is restricted to an ordered series of biconvex discs. The latter are formed originally, according to Kupffer (**13**), from " refringent particles " appearing in the plasma of the cells of the notochord, and increase in size, finally uniting (in Fam. *Ascidiidæ*) to form a more of less elastic hyaline axis. In *A. aspersa*, *A. mentula*, and *A. mammillata*, so far as we have observed, the latter stage is not attained until some time after the larva has been free, whereas in *A. scabra* it occurs even prior to hatching, although the hyaline axis may be discontinuous in places—i.e. broken up into several rod-like portions, this probably being an intermediate stage to complete fusion.

There is thus no readily to be observed essential difference in the structure of the larvæ, save perhaps with regard to stage of notochordal development at hatching, and this point is worthy of further investigation. There remains to be considered the question of larval size. Here again, as in the case of the eggs, there is apparently some slight seasonal as well as regional variation.

Length (μ). Means in brackets.

Larva.	Head (excluding papillæ).	Tail.	Head and Tail.	No. of Specimens.
A. aspersa	270–310 (284)	575–675 (620)	845–985 (904)	8
A. scabra (Aug.)	250–275 (262)	700–750 (725)	950–1020 (987)	9 { Plymouth / North Sea / North Scottish Coast
A. scabra (Aug.)	230–250 (238)	575–620 (597)	805–870 (835)	3 (Islay)
A. scabra (Oct.)	175	525	700	1 North Sea
A. mentula	175–250 (204) *(133)	450–650 (544)	625–900 (748)	4
A. mammillata	180–190 (185) *(208)	520–550 (535) *(522)	700–740 (720) *(730)	2

* According to Lohmann (**16**).

From these data it is obvious that the dimensions of the larvæ of *A. scabra* vary sufficiently to cover almost completely the range of variation in size of larva in the other three species. Probably the only safe deduction is that the larvæ of *A. mentula* and *A. mammillata* are rather smaller than those of *A. aspersa* and *A. scabra*.

Conclusion.

The foregoing investigation of the features of *A. aspersa* and *A. scabra* clearly establishes the fact that, by means of certain of these features, these two forms can always be distinguished from each other. The specific points of difference are found in (1) the relationship holding between number of tentacles and longitudinal vessels and (2) the nature of the egg. Almost, but not absolutely specific, are the differences in the following characters: (3) shape of the dorsal tubercle; (4) average size; (5) nature of test, and (6) position of the atrial aperture relative to the branchial aperture. The following are features of less specific value: (7) geographical and bathymetric distribution and (8) extent of area of attachment to the substratum. Little or no specific value can be attached to (9) colour; (10) number of stigmata per mesh of the branchial sac; (11) distribution of the renal cells, and (12) size and structure of larva (if the possible more rapid notochordal development in *A. scabra* be excepted).

Characters which did not in this case appear to call for special investigation were the nature of the musculature in the mantle and the details in connection with the alimentary tract, the dorsal lamina and the gonads.

Any or all of these characters might, however, be of specific importance where other species are concerned.

2. Variation in other Species of Family Ascidiidæ.

A less extensive material relating to these well-defined species was examined.

(1) Ascidia mentula.

From 35 collections of this species 53 individuals were selected for dissection. This material was obtained from various parts of the Scottish coast between the Moray Firth and Loch Fyne, over a general depth range of 17–60 metres. No justification was found for differentiating the results according to area, and they are here presented in combination.

A. mentula, along with the remaining species of the Family, is distinguished from *A. aspersa* and *A. scabra* by the possession of typical free papillæ on the branchial sac. This is a constant feature, and need not be further dealt with. Along with *A. conchilega* and *A. mammillata* this species can be distinguished from *A. virginea*, *A. obliqua*, and *A. prunum* by having the branchial sac prolonged behind the viscera. This feature also is constant. From *A. conchilega* it has been distinguished on account of having the nerve ganglion situated on the mantle at a point distant from the dorsal tubercle by about one-fourth (as compared with one-sixth) of the body-length, by possessing small instead of large renal cells, and a smooth as compared with a papillate test surface. Secondary branchial papillæ have been stated constantly to occur, whereas in *A. conchilega* they may occasionally be absent. These points of distinction therefore require special investigation. From *A. mammillata* the species is sufficiently distinguished by the occurrence in the latter of a recurved branchial sac and a peculiar modification of the dorsal tubercle.

(A) *Distribution.*—*A. mentula* resembles *A. aspersa* in that while it extends northwards along the west Scottish coast (being found even in the northern part of the Moray Firth), it is generally absent from the east coast of the British Isles.

(B) *Size.*—From 5–18 cm. in length.

(C) *Attachment.*—This is usually effected by means of a small area at the left posterior part of the body. There are however all variations from this slight attachment to complete fixation by the side or even, in a crevice, by a portion of both sides. In our material 3 specimens were attached by almost the whole side, 8 by from two-thirds to three-fourths, 12 by rather more than one-half, 9 by one-half, 4 by rather less than one-half, and 17 by about one-third of the side. (Two of the latter were attached not basally, but by a section of the mid-portion of the side.) As

has been the case with regard to other species, differences in mode of attachment have in the past been made part of the basis for splitting this clearly-defined species into several species (see, e.g. Alder and Hancock's monograph, in which 8 species and 1 variety, all referable to *A. mentula*, were described).

(D) *Colour.*—This varies from a horn-coloured or milky white tint in young specimens to a much darker tint in older specimens, which range in colour from greenish white through red to dark brown.

(E) *Test.*—This is thick, cartilaginous, and smooth in young individuals, but becomes more or less wrinkled or roughly furrowed (though not tuberculated) in older animals.

(F) *Position of Apertures.*—The atrial aperture is usually described as lying at a point distant from the branchial by from one-half to two-thirds of the body-length. In the specimens dealt with here, however, the interval was rather less than one-half in a minority of 9 cases. Of the remainder 10 showed an interval of one-half, 32 of rather more than one-half and only 2 of two-thirds.

The results in the case of *A. conchilega*, which rather resembles *A. mentula* in this character, may be given here. Out of 16 specimens, 4 showed an interval of rather less than two-thirds, 7 of rather more than one-half, 3 of one-half, and 2 of rather less than one-half. These two species therefore agree with regard to this feature, and may by its means be distinguished from all the other common species of Family *Ascidiidæ* except *A. mammillata*.

(G) *Branchial Stigmata.*— The number of stigmata per mesh of the branchial sac of *A. mentula* is usually given as being 6 or 7. Counts made on the above material showed that specimens from the region N. Moray Firth–Orkney possessed from 4–7 stigmata between sizes 4–8 cm., and from 5–8 between sizes 8–18 cm. There was thus an increase of only one slit in the larger as compared with the smaller sizes. Individuals from the N.W. and W. Scottish coasts possessed from 4–6 and 4–7 slits in the smaller- and larger-sized animals respectively. That is, accompanying increase of size, there was practically no increase in number of stigmata. In this species therefore it seems that the number is fairly constant (4–8) and that there is little increase with increasing size of individual. Slight differences may arise with change of area since the individuals of the Orkney region appear consistently to form about one more slit per mesh than do those of the west coast.

(H and J) *Number of Tentacles and Longitudinal Vessels.—A. mentula* is generally recognised as having from 20 80 (mostly about 40) tentacles. In our material the number was found to vary from 33–100 in individuals ranging in size from 4–18 cm. The average number was about 65. All rudimentary tentacles were as usual included in the counts. The number

of tentacles did not obviously increase with increasing size of animal. The number of bars did, however, so increase. Thus if number be plotted against size as in Figure 1 the loci fall in the neighbourhood of a curve rising gently from a point representing about 64 bars (at size 4 cm.) to one representing about 90 bars (at size 18 cm.). The entire range of number of bars is from 60–105.

(K) *Shape of Dorsal Tubercle.*—In descriptions of this species the tubercle is usually said to be irregularly rounded or horse-shoe shaped. Of the specimens dissected 47 out of 51 could be said to possess U-shaped tubercles. Of these 15 had the left horn and 11 both horns incoiled. These are evidently the usual shapes formed in Scottish waters. Of the remainder 8 were simply U-shaped, 7 had the right horn incoiled, 3 the left horn turned outwards, 2 both horns turned left, and one both turned out. It is noteworthy that a minority of 4 tubercles were (as is typically the case in *A. aspersa*) broadly ovate, with both horns incoiled.

(L) *Distribution of the Renal Bodies.*—These cells are of moderate size and form a coating over the viscera.

(M) *The size and nature of the eggs and larvæ* were discussed under *A. aspersa* and *A. scabra.*

Reference remains to be made to two characters which are used to form part of the means of distinguishing *A. mentula* from *A. conchilega.*

(N)—Although in the first-named species there are almost always, and in the latter rarely, secondary papillæ lying between the primary papillæ of the branchial sac, the distinction is liable to fail unless a large tract of the sac be examined.

(O)—On the other hand, the relative distance of the dorsal tubercle from the ganglion (this distance being expressed as a fraction of body-length) may definitely be used as a specific point of difference between these species. For *A. mentula* the fraction is usually stated to be one-fourth, and in the 51 specimens examined an average of 0·26 was obtained. The lower and upper limits were, however, 0·17 and 0·37. In *A. conchilega* the variation was found to be from 0·10–0·18 (average 0·14, or one-seventh, as compared with one-sixth found by previous workers). There is thus practically no overlap into the range of variation found for *A. mentula.*

(2) Ascidia conchilega.

Out of 25 separate collections of this species 16 specimens only were taken for dissection. The latter were obtained from the northern North Sea, the Faroe–Shetland Channel, the Minch and Eddystone, being taken in depths ranging from 98–229 metres.

(A) *Distribution.*—This is not yet very fully known, but appears to resemble that of *A. mentula,* though with a rather more northerly bias. Although the lower depth limit has hitherto been regarded as being

360 metres, this species has occurred in a recent Scottish Collection from a depth of 1104 metres in the Faroe–Shetland Channel, whence it has not hitherto been obtained.

(B) *Size.*—The specimens here dealt with ranged in size from 10–45 mm., but a size of 55 mm. has been recorded. The species may therefore readily be distinguished from *A. mentula*, which is seldom taken at a size of less than 55 mm.

(C) *Attachment.*—In 14 of these specimens the attachment was by almost the complete side, and in one individual by the complete side. In the remaining case, however, less than half of the side was fixed. This species is described as being fixed by the whole length of the left side, and this is probably usually, though not invariably, the case.

(D) *Colour.*—There is no obvious pigmentation. The animals have a more or less pale greenish tint.

(E) *Test.*—This is rather thin and cartilaginous, and has numerous minute tubercles on the free surface.

(F) *Position of Apertures.* –This has been dealt with under *A. mentula*.

(G) *Number of Stigmata in a Mesh of the Branchial Sac.*—In animals of size 10–13 mm. this number was from 2–3, but rose to from 3–4 at size 15–28 mm. and to from 5–7 at size 34–46 mm. The number therefore tends to increase as the individual grows.

(H, J) *Relative Number of Tentacles and Longitudinal Vessels.*—The number of tentacles was found to vary from 44–125, and did not appear to be dependent on the size of the animal. There are in these species three distinct rows of tentacles of different size—a feature absent from or not so clearly seen in the other species of the Family.

On the other hand, the number of bars was found to increase steadily with the size—from about 34 at a size of 10 mm. to about 54 at a size of 45 mm.

(K) *Shape of Dorsal Tubercle.*—In this species the tubercle is almost always horse-shoe shaped. Modifications of this simple fundamental shape are practically absent. In one individual, however, the right-hand horn of the tubercle was found to be incoiled, and in another the horns were prolonged.

(L) *Renal Bodies.*—These are particularly large and obvious, though of varying degrees of transparency. They are practically confined to the region of the viscera. Their arrangement over the stomach often imparts a ridged appearance to that organ.

(M) *Eggs and Larvæ.*—The egg of this species was described under *A. aspersa*. The larva was not obtained.

It is seen that *A. conchilega* may easily be distinguished from *A. mentula* by determination of the nature of the following characters : Bathymetric distribution, test, distance of dorsal tubercle from nerve ganglion, size

of renal cells, frequency of occurrence of intermediate papillæ in the branchial sac, and number of longitudinal bars (in the former species only about 54 occur at a size of 45 mm. ; in the latter 64 at a size of 40 mm.).

Differences in the shape and course of the gut are not discussed here, since the latter is often obscured by the overgrowth of renal bodies and gonads. Features of little or no specific use in separating these species are the nature of the attachment, the colour, the relative distance apart of the apertures, the number of gill-slits and tentacles, the shape of the dorsal tubercle, and the size and structure of the egg. In cases, however, in which the number of tentacles exceeds 100 the probability will be that a specimen of *A. conchilega* is being examined.

(3) Ascidia mammillata.

This species is easily identified. It has been recorded from as far north as the Clyde Estuary but has not recently been found there. With the exception that its northerly limit is probably the region Irish Sea–Firth of Clyde, this species is distributed over much the same area as is *A. mentula*. Three specimens from Plymouth were dissected. The surface of the test is mammillated, and this feature, added to those of the reflexed branchial sac and the peculiar structure of the dorsal tubercle, sufficiently distinguishes the species. The dorsal tubercle proper is a scarcely to be discerned gutter-shaped opening, very far distant from the nerve ganglion. More prominent, however, are the very numerous bright orange secondary openings situated all along the course of the neural canal.

The following are other features of the species, with the relative observations (in brackets) made upon the three specimens : Size from 5–13 cm. (5, 5½, 8). *Attachment* diagonally by the base (by more than one-third, by one-half, and by more than one-half of the length of the side). *Colour* of test opaline white or yellow, of mantle dark pink to blue. *Apertures* (atrial nearly one-half of the body-length distant from the branchial). *Number of stigmata per mesh* (from 5–7 in these medium-sized specimens). *Branchial papillæ* (as in *A. mentula*). *Tentacles*—of three sizes, and about 48–60 in number (56, 74, and at least 65 were counted in these specimens) ; the number of *longitudinal bars* was 78, 87, and 102 respectively, and appears therefore to increase with size of animal ; the egg and larva have already been referred to ; the renal cells are abundant but confined to the visceral region.

Of these features only one—the colour of the mantle—can be said to distinguish this species from *A. mentula*, which is the only form likely to be confused with it.

There remain to be considered three boreal species of Family *Ascidiidæ*. These are distinguished from (1), (2), and (3) by having no prolongation

of the branchial sac beyond the viscera ; further, the dorsal tubercle lies close to the ganglion, and the apertures are not more than one-third of the body-length apart. In all three of these characters they resemble *A. aspersa* and *A. scabra*, from which they differ in having real free papillæ in the branchial sac.

(4) Ascidia obliqua.

From 18 separate collections of this northerly deep-water species 33 specimens were taken for dissection. Of these 8 were from the Faroe–Shetland Channel and 25 from Faroe.

(A) *Distribution.*—Like *A. prunum*, this species is limited to Arctic and north Boreal regions, but may extend its range slightly into northern British waters by way of the deep channels whose water is partly of Arctic origin. The record given by Herdman (**8**) from Lamlash Bay, Firth of Clyde, should probably have been referred to another species. (We have found in a collection from Ettrick Bay an individual of *A. conchilega* which rather resembled *A. obliqua* outwardly.) Both *A. prunum* and *A. obliqua* extend their range into deep water, the former to nearly 400 metres' depth, and the latter (according to a new Scottish record from the Faroe–Shetland Channel) to 1250 metres. *A. obliqua* may in fact be said to be the northern deep-water counterpart of *A. scabra*, being quite the commonest Ascidian species in, e.g., that Channel. *A. virginea* is distinguished from these two more northerly species by having a south-Boreal distribution (from the Mediterranean to Faroe). That is, it is commoner in the south, and solitary specimens only are to be taken from the northern North Sea and the coastal waters of west Scotland.

(B) Size of *A. obliqua.*—From 25–75 mm. The individuals dealt with here ranged in size from 30–73 mm.

(C) *Attachment.*—*A. obliqua* is attached rather diagonally by a large circular area which includes the base and part of the left side. When removed from the water the animal, which has a flaccid body, sags down around this base. This is a distinguishing feature of the species.

(D) There is no pronounced pigmentation, the test being merely horn-coloured, though occasionally somewhat rugose in older individuals.

(E) *The test* is fairly thin and soft, with smooth or wrinkled surface. The species often adheres loosely to other Ascidians.

(F) *The apertures* are set not far apart upon conical and rather deeply lobed tubes. In the specimens examined their distance apart was one-third of the body-length in 19 cases, rather more than one-third in 10 cases, and rather less than one-third in 4 cases.

(G) *The number of stigmata* per mesh of the branchial sac is usually stated to be from 3–6. In the above material the number was from

3–5 in the smaller and from 4–6 in the larger specimens. There are no intermediate papillæ in the sac of this species.

(H, J) *The number of tentacles* varied from about 30–75, and apparently rises for some time with increasing size of individual, but becomes more or less constant after a size of about 50 mm. is attained.

The number of longitudinal bars increased gradually with the size of the animal from a minimum of about 65 (at size 30–50 mm.) to a maximum of about 104 (at a size of from 40–70 mm.).

(K) *The dorsal tubercle* is very large for the size of the animal and was found always to be elongate—U-shaped. The horns are prolonged but never coiled to any great extent. This is one of the species in which the size and shape of the tubercle are distinctive.

(L) *The renal cells* are not large. They are finely felted over but not beyond the visceral region.

(M) *The egg* of the species is, as has been stated above, probably the largest of any formed by the species of Family *Ascidiidæ*.

The larva has not been obtained.

(5) Ascidia prunum.

This species is at best rare even in north British waters. It has been taken by Scottish research vessels only off the coast of Iceland. From the collections from that coast 3 specimens only were examined for the purposes of this investigation. These were from 10–45 mm. in length, but a size of 70 mm. can be attained by this species. The attachment was by almost one-half of the left side in two of the cases and by the whole side in one. The apertures were separated by a distance equal to rather less than one-third of the body-length in two individuals, and rather greater than one-third in one individual. The test, as in *A. obliqua*, is usually thin and semi-translucent, but is never flaccid, and may become moderately thick. In young individuals the surface is smooth, but later it becomes a little wrinkled. Minute papillæ may be present. In the branchial sac intermediate papillæ are almost always present. There are stated to be from 3–5 stigmata per mesh of the sac in smaller and from 6–7 in older animals. (In these three specimens rather more were found, viz., from 5–6 at a size of 10 mm. and of 35 mm., and from 7–8 at a size of 45 mm.) The number of tentacles is usually given as being about 50, of three sizes. (In these specimens the number varied from 17 in the smallest to approximately 95 in the largest individual, and probably increases with size of animal, at least for some time.) The number of longitudinal bars rose similarly from 19–73 (from 40–70 is the range of variation given in the descriptions by other workers). The dorsal tubercle in its simplest form is merely a crescent-shaped slit, but it may also be half-moon or horse-shoe shaped. In the latter case one or both

horns may be bent inwards. Large renal bodies are distributed over the region of the viscera and may even extend slightly beyond the latter.

A. prunum therefore differs from *A. obliqua* in being more completely attached, and in the nature of the test and habit. The renal cells are larger, but the egg is probably smaller than in the latter species. The dorsal tubercle is smaller and of different shape. There are fewer longitudinal bars in the branchial sac, in which, however, intermediate papillæ are present.

Features of little or no distinctive value are the geographical distribution, the size, the colour, the distance apart of the apertures, and the number of stigmata and of tentacles.

(6) Ascidia virginea.

This species is rather rare. Out of 12 collections containing it we have taken only 8 specimens for dissection. Three of these were obtained from Plymouth and 5 from points on the west Scottish coast between the Clyde and Shetland. The total size-range was from 27–58 mm., but this species can reach a size of 75 mm. In shape the body is elongate—quadrilateral, tapering slightly towards the anterior end. In one specimen from Shetland the test was produced into a stalk, with the result that the animal was pear-shaped. One Plymouth specimen was attached by the entire side to a shell. This modification of the usual basal form of attachment has not, as far as we are aware, been hitherto observed, and forms an analogy with the depressed forms of *A. aspersa*, *A. scabra*, and *A. mentula*. Of the other specimens 6 were attached basally and one by two-thirds of the left side. This species is usually capable of being identified by the nature of the test, which is glabrous, rather gelatinous in texture, semi-transparent, and of a delicate pinkish hue. The apertures are quite sessile, the atrial aperture lying at a distance of rather less than one-third of the body-length from the branchial. (In 5 cases the interval was less than one-third, in 2 it was one-third, and in one—the depressed form—rather more than one-third.) In the meshes of the branchial sac the number of stigmata is stated as varying from 6-8. In our specimens the number ranged from 4–6 in the smaller and from 6–9 in the larger individuals. The crowded tentacles are often extremely difficult to count, especially since there are many minute ones. The number found exceeded that usually quoted, there being about 60 of the first order and probably about an equal number of a lesser size, some of which are mere papillæ on the tentacular collar. The minimum number of tentacles is probably about 60, and the maximum upwards of 120, but there is no obvious increase in the number as the size of the animal increases. As is more usual, however, the number of longitudinal bars

does so increase, regularly in the case of this species from about 35 at a size of 27 mm. to about 54 at a size of 58 mm. The dorsal tubercle is simply U-shaped (in two cases, however, the left horn was incoiled and in one the horns were prolonged after the fashion of those of the tubercles of *A. obliqua* and the Dogger Bank specimens of *A. scabra*). The renal bodies are not prominently seen. Secondary papillæ are rare or rudimentary in the branchial sac. The egg is moderately large.

The chief features distinguishing *A. virginea* from *A. obliqua* and *A. prunum* are therefore the nature of the distribution, of the test, and of the shape of the body. The number of longitudinal bars is more restricted. An additional distinction lies in the shape of the gut, since in this species the anal portion runs directly forwards so that the anus is considerably higher than the uppermost portion of the loop of the intestine (Plate 7, Fig. 4). In the other two species the anus is at approximately the same height as, or is actually lower than, that portion of the intestine.

A. TRITONIS (Herdman, 1883), the remaining Boreal species of Family *Ascidiidæ*, has been recorded from various parts of the slope of the N.W. European continental shelf, viz., to the south-west of Ireland and of Faroe and to the south of the Wyville–Thomson Ridge, at depths ranging from 840 to 1210 metres. It has not occurred in recent Scottish collections from either slope of the latter Ridge, and appears to be confined to very deep water in a limited area. As described by Herdman (**9**) and by Hartmeyer (**7**), this form would appear to have many features in common with *A. conchilega*. It reaches a much greater size, however, the range being from 5–17 cm. It is noteworthy that our new record of *A. conchilega* from a depth of 1104 metres does away with any absolute bathymetric distinction being made between the two species. This record was made, however, in the Faroe–Shetland Channel, on the north (or cold-water) side of the Ridge, where the temperature is below zero, whereas the temperature was found to vary from $5\frac{1}{2}$–$8\frac{1}{2}$ °C. on the south side of the Ridge whence *A. tritonis* has been obtained. It is suggested here, nevertheless, that proof is required that the latter species is not merely a large form of *A. conchilega*. Hartmeyer, from a study of about 50 specimens of the former species, revised Herdman's description. The outstanding features appear to agree in most details with those of the latter species. The branchial sac extends beyond the gut, the course and appearance of which, judging by Herdman's illustration, agree with those found in *A. conchilega*. The ganglion is separated from the dorsal tubercle by a distance equal to approximately one-sixth of the body-length. There are small papillæ on the test, which is cartilaginous in texture. The

attachment is by from one-half to two-thirds of the left side, and the atrial aperture is separated from the branchial by a distance equivalent to from rather less than one-half to two-thirds of the body-length. There are about 3 stigmata in a mesh of the branchial sac of smaller, and 5 in the sac of larger individuals. (The number in *A. conchilega* rises to a maximum of from 5–7, so that here there may be a point of difference.) Branchial papillæ are present but secondary papillæ are absent. (In *A. conchilega* the latter are occasionally present, but we have found that this is not quite a specific feature, since the formation of secondary papillæ is—in some species at least—the prelude to the formation of transverse vessels between them, with the result that the original mesh is divided transversely into two meshes. In other words, the more fully developed the branchial sac is—e.g. in *A. mentula*, whose sac may or may not show secondary papillæ of a lesser size than primary—the less chance there is of finding in it intermediate papillæ.) The tentacles are very numerous—from 80–100 or more. The number of longitudinal bars exceeds 100. In *A. conchilega* the number rose steadily with size of animal to about 54 at a size of from 4–5 cm., and might conceivably be much greater in individuals of double or treble this size. In this species, as in *A. tritonis*, the dorsal tubercle is simply horse-shoe shaped, with rare modifications.

We found that in *A. conchilega*, while the number of tentacles did not appear to be dependent on the size of the animal, the number of bars did so. This would account for the agreement in the number of tentacles of the two species and the disagreement in the number of bars. The presence or lack of intermediate papillæ in the sac may prove to be a constant and specific feature whereby these species may be distinguished. Beyond this and the slight difference in number of branchial stigmata per mesh there seems to be little to go upon other than the difference in the maximum size which the animals may attain. It would apparently be very difficult to state to which species individuals of, e.g., 5 cm. in length belonged.

From the Key to the Boreal *Ascidiidæ* given below *A. tritonis* has been omitted for these reasons and since the form is not likely to be encountered in water of moderate depth.

3. A Suggested Key to the Boreal Species of Family Ascidiidæ.

It is hoped that the foregoing does something to bring into clearer prominence those features which may reliably be used in the classification of the species of this Family. Our experience has been that in nearly every case the species can be recognised from its external appearance. That is, the number of possibilities is first of all narrowed down by such

a character as the distance apart of the apertures (the corollary of which in internal structure is the distance apart of nerve ganglion and dorsal tubercle) ; it is further narrowed down by consideration of the nature of the test and the habit. The size of the animal and its mode of attachment to the substratum are of less importance, and little reliance need be placed on the colour. The ability to identify a species from its external appearance is, however, and must remain, the outcome of knowledge gained by dissection of most if not all of its forms of variation—including variation in size, which may or may not entail variation in such characters as the number of tentacles and longitudinal bars, of the stigmata in a mesh in the branchial sac, and even in the presence or absence of secondary branchial papillæ. Internal evidence of first importance is afforded by the presence or absence of a prolongation of the branchial sac behind the region of the viscera, and of true papillæ in the sac. The shape and position of the dorsal tubercle are of importance in certain cases, but variation in the former character often occurs. Importance can seldom be attached to pigmentation of the mantle. The shape of the gut is often largely obscured by overgrowth of gonads and renal cells, but the terminal portion of the intestine can easily be seen, and its course and relative position afford in certain cases evidence of specific value. The size and distribution of the renal cells, while aiding an identification to be confirmed, are not of primary importance, and in this Family a study of the nature of the gonads—organs whose appearance is subject to change with season and with size of animal—is unnecessary for purposes of identification. Much information still remains to be gathered with reference to the precise limits of geographical and bathymetric distribution of certain of the species. In addition the developmental characters require further study ere full use can be made of them for systematic purposes.

It is suggested that the three existing genera of Family *Ascidiidæ* may be combined without entailing increased difficulty in the identification of the species or failure to give due weight to the known structural or developmental characters. The Family is not crowded with species, and the present division into genera is not a natural one. Genus *Phallusia* has been established to include a species to be distinguished from the others of the Family by having a modified type of dorsal tubercle and a reflexed portion of the branchial sac. But, as has been shown, this species resembles *A. mentula* and *A. conchilega*, and differs from the other species of the Family in having the branchial sac produced behind the gut and the dorsal tubercle placed at a considerable distance from the nerve ganglion. In addition, as far as we know them, both the eggs and the larvæ of these three species seem very similar. These deep-rooted resemblances would

appear to warrant the formation of a genus (Gen. *Phallusia*) were it desirable that a separate genus be formed, and seem far to outweigh the rather trivial differences upon which the existing definition of Gen. *Phallusia* rests. In all three species the typical form is attached diagonally to the substratum, the apertures are very widely separated, and with the possible exception of *A. conchilega*, the upper limit of whose size requires confirmation, a very large maximum size is attained. The diagonal attachment is necessary if the atrial aperture is to be kept clear of the substratum (Plate 7, Figs. 5–6), whereas, in the remaining species the typical form can well be erect since the apertures are rather closely approximated and anterior (Plate 7, Fig. 4). Only in *A. obliqua* is there a tendency to oblique attachment, and only *A. aspersa* reaches a size of over 10 cm., with an average size much smaller than that figure. Tubercle and ganglion are closely approximated and the branchial sac does not extend beyond the gut. These species therefore appear to form a natural group from which it is difficult to justify the separation of genus *Ascidiella* by reason of the lack of real branchial papillæ in the latter and the excessive straying of renal vesicles over the mantle surface. As has been said, the absence of papillæ is only a matter of degree (the appearance of the bars at certain stages of development and from certain angles, has led numerous observers to state that papillæ are to be seen, but on further study all have agreed that real free papillæ, projecting abruptly from the surface of the longitudinal vessels and not being mere thickenings of these vessels, are absent). The extension of the distribution of the renal cells beyond the viscera is simulated in a lesser degree in *A. prunum*. These points of difference are, by comparison, scarcely of generic value when balanced against the more deeply-set features of resemblance with *A. obliqua*, *A. virginea*, and *A. prunum*. Also, the implied diminutive in the term *Ascidiella* is misleading. While there might be considered grounds for the separation of these smaller forms into a genus apart from that which would contain the larger forms, the simpler course is suggested here of bringing together into one genus all the Boreal forms of the Family. To this procedure there appears to be no obstacle arising from the study of developmental characters, at least as far as this study has gone.

KEY TO GENUS ASCIDIA.

1.	Nerve ganglion adjacent to dorsal tubercle; apertures not more than one-third of the body-length apart; sac not prolonged beyond the viscera	2
	Ganglion at a considerable distance from the tubercle; apertures much more than one-third of the body-length apart; sac prolonged beyond the viscera	6
2.	Typical free papillæ absent from the sac	3
	Typical free papillæ present in the sac	4

3.	With more longitudinal vessels than tentacles; test papillate or pustolose; dorsal tubercle transversely ovate; ova with large follicle cells	*A. aspersa.*
	With fewer longitudinal vessels than tentacles; test smooth or wrinkled, with papillæ absent or confined to the region of the siphons; dorsal tubercle U-shaped	*A. scabra.*
4.	Anal portion of gut running directly forwards; anus considerably higher than upper bend of the intestine; body of elongated quadrilateral shape, tapering slightly anteriorly	*A. virginea.*
	Anus at approximately the same height as, or lower than, the upper bend of the intestine; body more or less roundish	5
5.	Intermediate papillæ absent from sac; gut of limited size; egg large	*A. obliqua.*
	Intermediate papillæ almost always present; gut extensive	*A. prunum.*
6.	Dorsal tubercle-proper small and irregular, but a great number of secondary openings are studded along the course of the duct from the neural gland; sac recurved upon itself	*A. mammillata.*
	Dorsal tubercle without secondary openings; sac not recurved	7
7.	Ganglion distant about one-sixth of the body-length from the dorsal tubercle; intermediate papillæ of the sac absent in places; renal cells large; test papillate	*A. conchilega.*
	Ganglion distant about one-fourth of the body-length from the dorsal tubercle; intermediate papillæ almost always present in the sac; renal cells small	*A. mentula.*

4. Synonymy.

(With chief reference to recent British Works).

Ascidia aspersa (Müll. 1776).

=*A. aspersa*+*A. truncata* (Herdman, 1881).

=*A. aculeata* (part)+*A. canina* (non. Müll.)+*A. Normani*+do. (var. *resplendens*)+*A. affinis*+*A. pustulosa*+*A. scabra* (var, *echinata*) (Alder & Hancock, 1905).

This species was described by Risso (**18**) as *Phallusia cristata* and later by others—including Roule (**20**)—as *Ascidiella cristata.* If, as Alder and Hancock thought probable, Müller's *A. aspersa* was a young stage of *A. scabra*, the specific name should probably be *A. cristata.* However, the present name has been much used, is expressive, and will probably be allowed to stand.

Ascidia scabra (Müll. 1776).

=*A. virginea*+*A. scabra*+*A. triangularis* (Herdman, 1881).

=*A. sordida*+*A. morei*+*A. scabra*+do. (vars. *albida*+*lævis*)+*A. elliptica*+*A. pellucida*+ ? *A. orbicularis*+ ? *A. vitrea* (Alder & Hancock, 1905).

=*Ascidiella scabra* (Roule, 1884).

Ascidia mentula (Müll. 1776).

=*A. lata*+*A. fusiformis* (Herdman, 1881).

=*A. mentula*+*A. robusta*+*A. rubicunda*+*A. rubrotincta*+*A. crassa*+*A. mollis*+do. (var. *carnosa*)+*A. plana*+*A. Alderi*+*A. rudis* (Alder & Hancock, 1905).

Ascidia conchilega (Müll. 1776).

=*A. depressa*+*A. plebeia*+*A. exigua* (Herdman, 1881).

=*A. depressa*+*A. Roulei* (part)+do. (var. *petricola*)+*A. Herdmanni* (Garstang, 1891).

=*A. producta*+? *A. inornata*+*A. depressa*+*A. elongata*+*A. aculeata* (part)+*A. amœna*+*A. plebeia* (Alder & Hancock, 1905).

Ascidia prunum (Kupff., 1875).

=*A. callosa* (Stimpson, 1854).

=*Phallusia prunum* (Kupff., 1875).

=*A. glacialis*+*A. prunum* (part)+*A. complanata* (Herdman, 1891).

Ascidia mammillata (Cuv., 1815).

=*A. mammillata* (Cuv., 1815).

= Do. (Alder & Hancock, 1905).

Ascidia obliqua (Ald. 1863).

=*A. obliqua*+*A. falcigera*+*A. mollis* (Herdman, 1891).

=*A. obliqua* (Alder & Hancock, 1905).

Ascidia virginea (Müll. 1776).

=*A. patoni* (Herdman, 1881).

=*A. venosa* (Alder & Hancock, 1905).

5. SUMMARY.

1. A detailed investigation is made into the identity of the forms recently grouped under Genus *Ascidiella*. Two species only have been found to be capable of being differentiated, and these have been re-included under Genus *Ascidia*.
2. The relative specific importance of certain developmental and body characters is discussed.
3. A new Key to the Boreal species of Family *Ascidiidæ* is suggested.

We are indebted to Mr. E. Ford, of the Marine Laboratory at Plymouth, for sending part of the living material utilised in this investigation. The thanks of one of us (S.T.L.) are due to the Fishery Board for Scotland for permission to work in their Laboratory at Aberdeen, and to the Trustees of the Carnegie Fund for Scottish Universities, from whom she has been in receipt of a Research Scholarship.

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PLATE 1.

Eggs of Fam. *Ascidiidæ*. (×110. Zeiss Camera Lucida.)

Fig. 1.—*Ascidia mentula*.

Fig. 2.—*Ascidia virginea*.

Fig. 3.—*Ascidia conchilega*.

Fig. 4.—*Ascidia mammillata*.

Fig. 5.—*Ascidia scabra* (2 hours after emission).

Fig. 6.—*Ascidia aspersa* (2 hours after emission).

Fig. 7.—*Ascidia prunum* (calculated size of chorion).

Fig. 9.—*Ascidia obliqua* (calculated size of chorion).

Fig. 8.—*Corella parallelogramma*. (Fam. *Rhodosomatidæ*.

Fig. 10.—Egg in oviduct of *A. scabra*.

Fig. 11.—Egg in oviduct of *A. aspersa*.

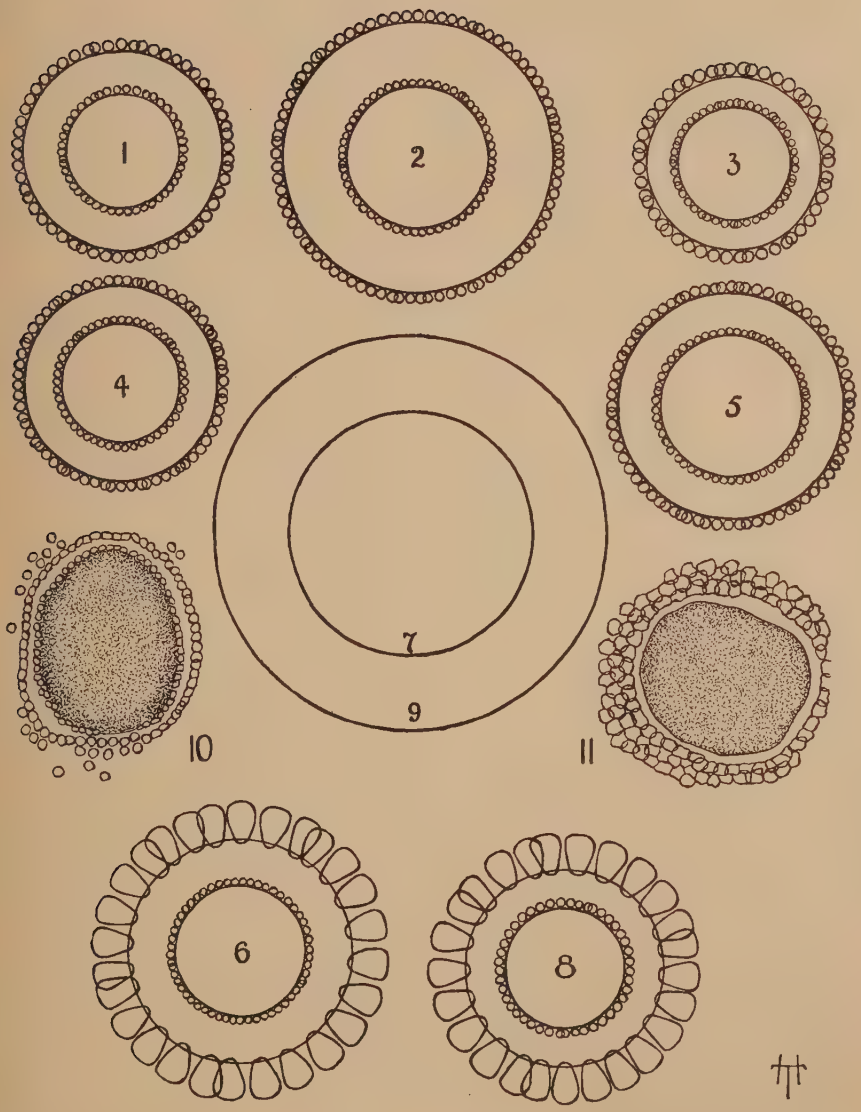
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PLATE 2.

Eggs of Fam. *Ascidiidæ*. (×110. Zeiss Cam. Lucida.)

Fig. 1.—*Ascidia aspersa* (Plymouth). 10 mins. after emission.

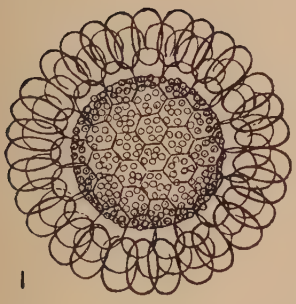
Fig. 2.—*Ascidia aspersa* (Plymouth). 2 hours after emission.

Fig. 3.—*Ascidia aspersa* (Islay). One day after emission.

Fig. 4.—*Ascidia scabra* (Plymouth). May.

Fig. 5.—*Ascidia scabra*, escaping larva (Aberdeenshire coast). August.

Fig. 6.—*Ascidia mammillata*, escaping larva (Plymouth).



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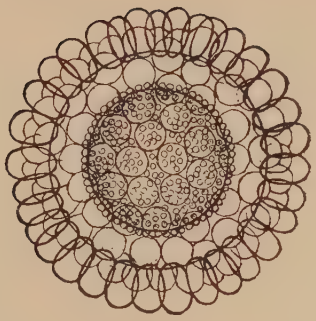

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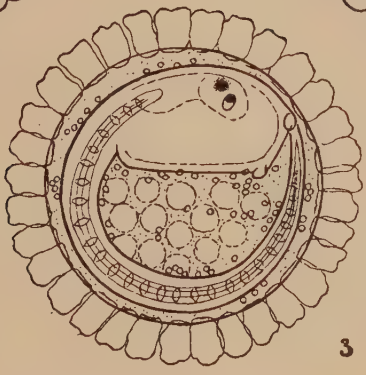

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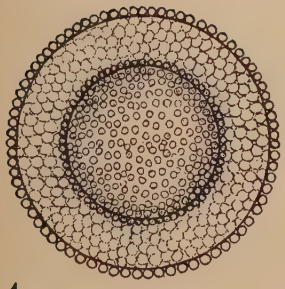

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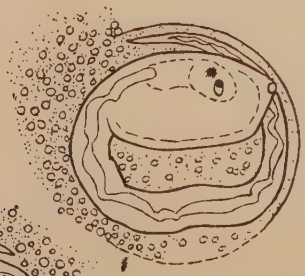

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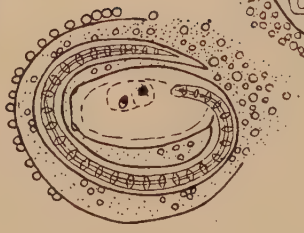

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PLATE 3.

Larvæ of Fam. *Ascidiidæ*. (× 104. Zeiss Cam. Lucida.)

Fig. 1.—*Ascidia aspersa* (Plymouth). Left side, oblique view.

Fig. 2.—*Ascidia aspersa* (Islay). Cf. Fig. 3, Plate 2.

Fig. 3.—*Ascidia scabra* (Plymouth, Aberdeen, Loch Eriboll, etc.). Cf. Fig. 5, Plate 2.

Fig. 3a.—*Ascidia scabra* (North Sea), an example of smaller late season (October) larvæ (cf. Fig. 3).

Fig. 4.—*Ascidia mentula*.

Fig. 5.—*Ascidia mammillata*.

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PLATE 4.

Types of Fixation and Habit in Fam. *Ascidiidæ*. (Seven-eighths natural size.)

Figs. 1–4.—*Ascidia scabra*.

Fig. 1.—Attached by side (Luce Bay).

Fig. 2.—A specimen from Firth of Clyde. The apertures are unusually wide apart.

Fig. 3.—Attached by base. A typically transparent specimen from Moray Firth.

Fig. 4.—Attached by base. Large Dogger Bank form.

Figs. 5–6.—*Ascidia virginea*.

Fig. 5.—Attached by base (normal method of fixation).

Fig. 6.—Attached by side (abnormal method of fixation).

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PLATE 5.

Types of fixation (*continued*).

FIG. 1.—*Ascidia aspersa*. Basal attachment (Plymouth).

FIG. 2.—*Ascidia aspersa*. Attached by side (Loch Indail, Islay). (Both figures natural size.)

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PLATE 6.

Dorsal Tubercles and Tentacles of *Ascidia aspersa* and *Ascidia scabra*.
(Zeiss Cam. Lucida.)

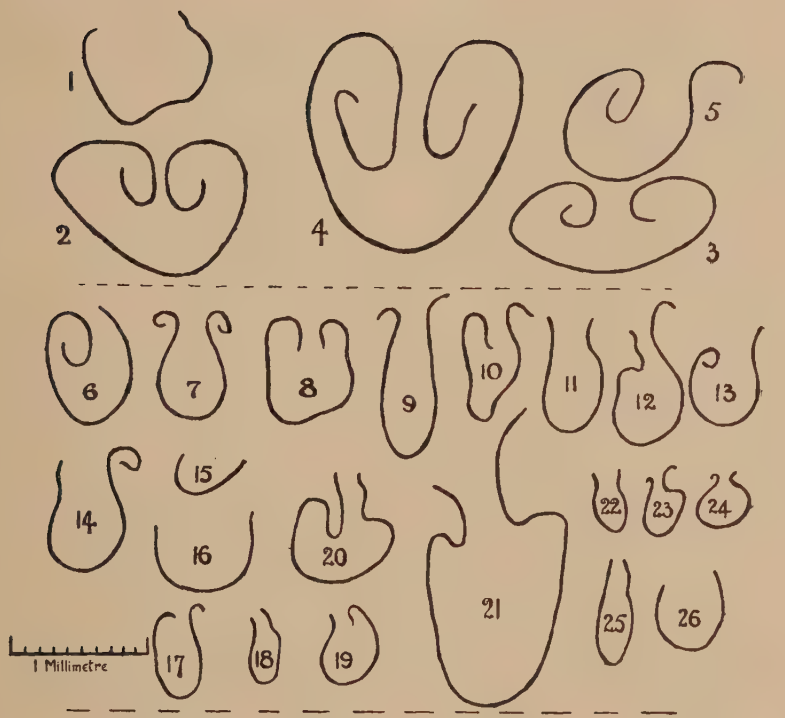
FIGS. 1–5.—Variation in form of Dorsal Tubercle in *A. aspersa*. Figs. 2 and 4 are the normal form; Fig. 1 (×3½ times ordinary scale) from a very small Plymouth specimen, 6 mm. in size; Figs. 2 and 3 are from Plymouth, and Figs. 4 and 5 from Loch Fyne specimens.

FIGS. 6–26.—Variation in form of Dorsal Tubercle in *A. scabra*. Figs. 6–16 from a random selection of specimens from the northern Moray Firth; Figs. 15 and 16 (×3½ times ordinary scale) from very small specimens (9 and 12 mm.); Fig. 17 from Loch Fyne;

Figs. 18–20 from Luce Bay; Fig. 21 from Dogger Bank; Figs. 22–26 from Plymouth.

FIG. 27.—A group of tentacles from *A. aspersa* (Plymouth), of size 40 mm. (×18).

FIG. 28.—A group of tentacles from *A. scabra* (Dogger Bank), of size 40 mm. (×18).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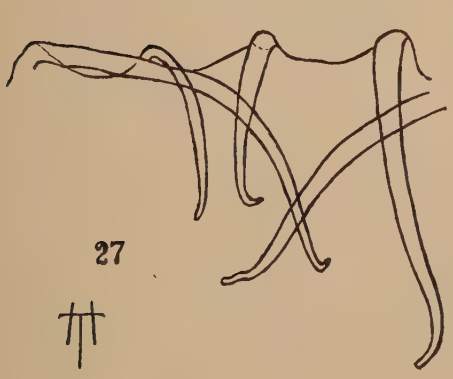
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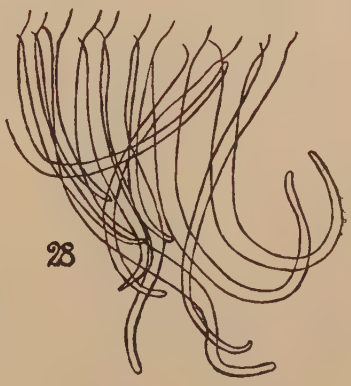
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PLATE 7.

FIGS. 1-3.—Portions of Branchial Sac.

FIG. 1.—*Ascidia aspersa*. Size 30 mm. (from Loch Indail, Islay), pseudo-papillæ only present (×60, Zeiss C.L.).

FIG. 2.—*Ascidia aspersa*. Size 8 mm. (from L. Indail).

a. =Knob-like vascular processes, simulating papillæ.

b. =A similar process linked up with its neighbour by a longitudinal bar (×110, Zeiss C.L.).

FIG. 3.—*Ascidia mentula*. Showing typical free papillæ (×60, Zeiss C.L.).

FIGS. 4–6.—Illustrating mode of attachment.

FIG. 4.—*Ascidia virginea*. Basal attachment is normal; apertures are well clear of substratum.

FIG. 5.—*Ascidia mentula*. Showing how basal attachment (none occurs in this species) would bring position of atrial aperture near to substratum.

FIG. 6.—*A. mentula*. Normal oblique attachment by side, atrial aperture free of substratum.

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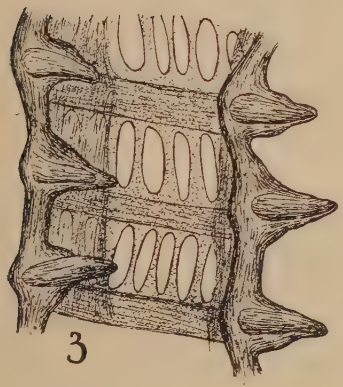

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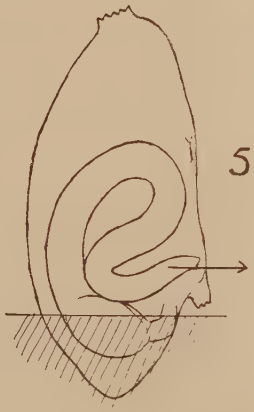

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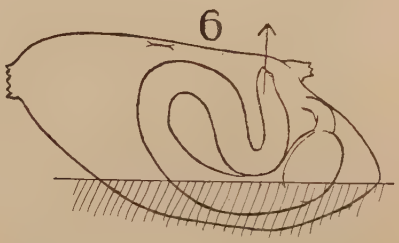

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PLATE 8.

A specimen of *A. scabra* showing double atrial siphon (Test removed).

Fig. 1.—From right side, showing musculature of mantle.

Fig. 2.—From left side, showing extension of renal cells beyond the viscera.

Both Figs. about three times natural size.

(Photographs by Mr. John McKenzie, of the Staff of the Fishery Board for Scotland.)

2.

1.

Some Abnormal Fishes Received at the Plymouth Laboratory.

By

E. Ford, A.R.C.Sc.,

Fisheries Naturalist at the Plymouth Laboratory.

With 8 Figures in the Text, and Plates I–V.

Introduction.

The object of this short paper is to describe a number of fishes exhibiting interesting abnormalities. It will be seen from the list given below that several of the specimens to be described were sent to the Laboratory on the personal initiative of gentlemen to whom I express my thanks. I am also greatly indebted to Dr. H. A. Harris and the staff of the X-ray Department of the Institute of Anatomy, University College, London, for the beautiful radiograms they supplied. Mr. C. H. Gill, Press Photographer, of Plymouth, photographed several specimens and also prepared prints for the illustrations.

	List of Specimens Described.	Date Received.	Source.
1.	Cod with Malformed Mouth*	May 10th, 1929	From steam trawler *Stormcock* landing at Plymouth, per P. Hawthorn, Esq., Inspector in the Public Health Department, Plymouth.
2.	A Pug-headed Herring	July 11th, 1927	From River Tamar, among catch of young clupeoids.
3.	A Fan-tailed Pilchard	Oct. 9th, 1928	From Newlyn, Cornwall. The specimen was noticed among salted pilchards being packed, and was forwarded by B. Dunn, Esq.
4.	Coalescence of Vertebræ in a Lemon-Dab (*Pleuronectes microcephalus* Donov.)	April 5th, 1929	From s.s. *Salpa*, trawling off Mewstone.
5.	A Deformed Stickleback (*Spinachia vulgaris* Flem.)	June 14th, 1928	From motor-boat *Gammarus*, trawling in Plymouth Sound.
6.	Abnormal Rays		
	6 A *Raia microcellata* Mont.	Jan. 1st, 1928	From s.s. *Salpa*, trawling on Eddystone Grounds.
	6 B *Raia clavata* L.	Feb. 24th, 1930	Landed at Plymouth market; caught in ray-net set in Bigbury Bay.
	6 C *Raia microcellata* Mont.	Feb. 25th, 1930	From s.s. *Salpa*, trawling in Bigbury Bay.
	6 D *Raia microcellata* Mont.	Feb. 25th, 1930	Ditto.
7.	Double-monster of the Spur-Dogfish (*Acanthias vulgaris* Risso)	Aug. 9th, 1929	From Newlyn, Cornwall, per R. Phillips, Esq., of Plymouth.

* I am indebted to Dr. R. H. Burne, F.R.S., and the Pathologist at the Royal College of Surgeons for their report on this specimen, and for the half-section of the fish figured in Plate I.

1. A Cod with Malformed Mouth.

The cod showing this abnormality measured 86·5 cm. in length and was in fairly good bodily condition despite its seemingly serious disability. The external features of the head are well shown in Text-Figs. 1 and 2. The snout projects beyond a curiously deformed lower jaw with a bulbous growth beneath, giving the head a most quaint appearance. The barbel (*b*), normally central, lies to the right of the bulbous growth, and what would normally be the left half of the lower jaw (*d*) is symmetrically arched across and beneath the upper jaw, presumably functioning as a

Text Fig. 1.—Head of Malformed Cod, viewed from right side. (Photo, C. H. Gill.)

whole lower jaw. The representative of the right half of the lower jaw is displaced and doubled back upon itself at a sharp angle with the left half. When first observed, the bulbous growth was ulcerated (Text-Fig. 2.*a*), leading one to suspect that it was pathological in character. The specimen was later sent to the Royal College of Surgeons where sagittal sectioning revealed the true state of affairs. Plate I from a photograph of the cut face of the left half of the head serves to illustrate the conclusions communicated to me by Dr. Burne. The protuberance is not a tumour, but is due to the downward bending of the tongue (Plate I, *t*), pushing the

intermandibular wall before it. Dr. Burne suggests that the condition is a congenital one ; i.e. want of growth of the lower jaw accompanied by full growth of the tongue, and the necessity for the tongue to find room. Instead of growing out of the mouth, the tongue has bent downwards, pushing the sub-mental wall before it. It occurs to me to suggest that the process described by Dr. Burne might have produced the end result if it came into operation subsequent to an injury to the lower jaw of a

Text Fig. 2.—Head of Malformed Cod, viewed obliquely from beneath. (Photo, C. H. Gill.)

a. lesion on ventral protuberance.
b. barbel, normally median in position.
c. original right-half of lower jaw.
d. original left-half of lower jaw, now functioning as whole lower jaw.

young normal individual. This fish certainly provides an excellent illustration of the ability to survive and grow under adverse circumstances.

2. A Pug-headed Herring.*

This specimen, 8 cm. in length, exhibits the features described by Gemmill (**2**, p. 48) and others under the name of "pug-head." Of this

* Dr. E. W. Gudger has recently published an interesting paper entitled "Pug-Headedness in the Striped Sea-Bass, *Roccus lineatus*, and in Other Related Fishes." Bull. Amer. Mus. Nat. Hist., Vol. LXI, March 31, 1930.

condition Gemmill says: "In typical examples the snout is markedly reduced in size, and curved in such a way that the anterior frontal region arches rapidly downwards just in front of the eyes. The latter are lessened in horizontal diameter and become oval in shape, having the long axis vertical. The lower jaw retains its usual size, and accordingly projects a considerable distance in front of the rest of the head." It can be seen from Text-Fig. 3 that the specimen conforms with this type description. In other respects the fish was normal and in healthy condition, its stomach being well-filled with recently-caught mysids. Gemmill refers to the interesting results of Knauthe who allowed breeding between males and females of *Leucaspius delineatus* v. Sieb. and *Cyprinus aphya ?*, which, though apparently normal, were derived from "puggy"

Text Fig. 3.—A Pug-headed Herring. Length 8 cm.

parents or grandparents. For instance, he found that a male and female Leucaspius, normal themselves though derived from "puggy" parents, provided in the offspring the large number of 50 out of 180 which "reverted" to the grandparental form. In later experiments with Cyprinus he was able to show that 6 of a total of 168 progeny were "puggy" like their great-great-grandparents although the intervening ancestors were all apparently normal.

3. A Fan-tailed Pilchard.

Measurements of this unusual specimen resulted as follows:—

Tip of snout to root of tail . . .	20·8 cm.
Tip of snout to end of mid-caudal ray .	22·0 cm.
Tip of snout to end of flukes . .	26·6 cm.
Greatest width of tail across flukes .	6·5 cm.

The relative size of the tail as compared with that of a normal fish of approximately the same length may be gathered from Plate II. Photography by X-rays (Plate II) reveals that the leaf-like expansion of the

tail is due to an excessive lateral branching and rebranching of the dermal fin-rays. At their proximal ends the latter retain their rod-like appearance and are in normal association with the endoskeletal elements of the caudal fin.

4. Coalescence of Vertebræ in a Lemon-Dab (*Pleuronectes microcephalus* Donov.)

The small Lemon-Dab in which this abnormality was observed measured 22·5 cm. from the tip of the snout to the end of the middle rays

Text Fig. 4.—A Deformed Stickleback (*Spinachia vulgaris* Flem.).
Upper: From above. Lower: From side.

of the caudal fin. It will be seen from the X-ray photograph (Plate III) that as the result of considerable co-ossification of vertebræ in the posterior half of the vertebral column the fish presents the contour of a top-knot rather than of a lemon-dab at the tail-end. The outer edge of the caudal peduncle is thus brought almost into alignment with the bases of the dorsal and anal fins in this region. Photography by X-rays has conveniently revealed the main features of this vertebral abnormality. Anteriorally the vertebræ appear to be normal and it is not until about the 18th vertebræ from the front that anything markedly unusual is

noticeable. At this point, however, there are signs of a dorso-lateral displacement of vertebræ, particularly evident in the next half a dozen vertebræ. It is as if these had become forced out of alignment as the result of undue compression along the vertebral column. Behind these obviously displaced vertebræ, the remainder of the series appear to be

Text Fig. 5.—Abnormal *Raia microcellata* Mont. Specimen 6 C, described in text.

co-ossified to form several unequal-sized blocks of fused vertebræ. Continuing the use of the "compression" analogy the appearance is as if pressure had been here so great that the centra had been forced to collapse together like the bellows of a camera, rather than being displaced dorso-ventrally. Nevertheless, the identity of the vertebræ still remains apparent in that the neural and hæmal spines are properly paired and normal in number, representing a total of 47 (or 48 ?) vertebræ exclusive of the caudal end-piece.

5. A Deformed Sea-Stickleback (*Spinachia vulgaris* Flem.)

This little fish of 8 cm. in length was alive and active when brought to the Laboratory and presented a most odd appearance while swimming in an aquarium. Viewed from the side (Text-Fig. 4) it showed a marked kink in the tail region posterior to the dorsal fin. Seen from above, it exhibited a sinuous formation of the body. Photographs by X-rays show that the vertebral column follows the lines of the kink and waves of the body but that the vertebræ are normal in character. This instance adds another to the long list of references given by Gemmill (**2**, p. 52) to cases of twisted bodies with or without associated coalescence of vertebræ. Gemmill refers to examples of twisting or curvature without coalescence of vertebræ (i.e. of the class to which the present stickleback belongs) in a sole and in a codfish, described by Howes (**4**) and Storrow (**6**) respectively. In both there were five sinuosities.

Text Fig. 6.
Vertebral skeleton of specimen 6 C, described in text.

6. Abnormal Rays.

6 A. A small female of *Raia microcellata* Mont., measuring 26 cm. across the disc, was found to show fin abnormalities on the right side. The pectoral was separated from the head by a deep triangular notch involving two gill-clefts. The posterior lobe of the right ventral fin was also divided into two by a somewhat shallower notch. These features are shown in Plate IV, 6 A.

6 B. A mature male of *Raia clavata* L., measuring 51 cm. across the disc, had the pectorals of both right and left sides separated from the head (Plate IV, 6 B), although in this case the separation did not extend so far back as in specimen 6 A, nor was there any accompanying abnormality of the ventral fin.

There are numerous references in literature to rays in which the pectoral fins are not adherent to the head, more commonly, perhaps, to cases in which both fins are free as in specimen 6 B. Bashford Dean (**1**, p. 620)

cites the genera Hieroptera and Propterygia as being originally described as specific forms although now known to be rays of this peculiarly abnormal type. He also credits Gesner (**3**) with the first known account and figure in 1556, and gives a list of references to published accounts in later times. Williamson (**8**, p. 64) advances reasons against the explanation of these abnormalities as the result of cutting by fishermen, pointing out the exactitude of the separation along the dividing-line between the snout and the fin on either side. Johnstone (**5**, p. 188) suggests that the malformation is due to the assumption, during development, by the propterygia, of a position which is more nearly related to the primitive position of the Elasmobranch pectoral fin-structures than the latter normally occupy in skates and rays. The fact that in specimen 6 A the posterior lobe of the ventral fin is also bifurcated adds interest to the question of the causation of such abnormalities in rays.

6 C and 6 D. By an odd coincidence two male rays of the same species (*R. microcellata* Mont.), taken in the same haul of the otter trawl,* showed sinuous twisting of the vertebral column.

6 C. This specimen measures 49 cm. across the disc, and the very abnormal condition of the tail is apparent from Text-Fig. 5 reproduced from a photograph. The appearance of the vertebral column (prepared by boiling and cleaning away the flesh) is shown in Text-Fig. 6. Attention is drawn especially to the pronounced " elbow " at (*l*), where in addition to the action of a wave of lateral displacement there is marked tortion in a clockwise direction through an angle of almost 90°. This twisting is, however, quite local to loop (*l*), the tail posteriorally retaining its normal dorso-ventral position although, of course, exhibiting the laterally-sinuous condition. The " elbow " at (*l*) was quite flexible, so that one imagines that the fish when alive could freely swing the tail posterior to it.

6 D. The second specimen, not figured here, was 48 cm. across the disc and more normal in appearance. The vertebral column was quite normal from the head backwards to the level of the distal ends of the ventral fins, but at this point there was one well-marked lateral looping, followed by a second in the region of the dorsal fins. There was nothing comparable to the tortion shown in specimen 6 B, nor, one thinks, was the fish unduly affected in its tail-action during life.

7. Double Monster of the Spur-Dogfish (*Acanthias vulgaris* Risso.)

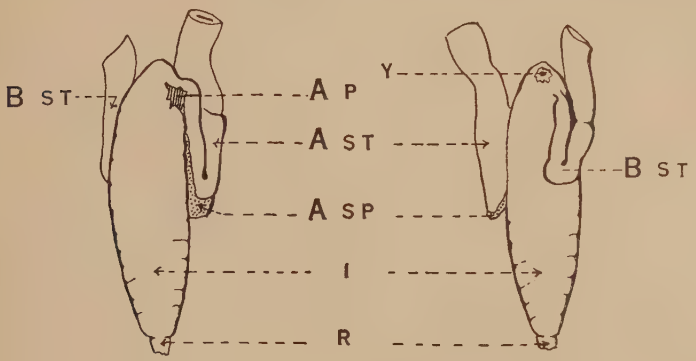
This specimen was first noticed among the embryos in the offal from dogfish being prepared for market at Newlyn. It was forwarded to

* My colleague, Mr. G. A. Steven, was aboard the *Salpa* on this occasion and brought back the specimens.

Plymouth in methylated spirit, curled up in a rather small bottle, and apart from the lack of the yolk-sac was entire.

External Features.

In size and development the monster may be likened to the union of two embryos between 13 and 15 cm. in length which had grown at the expense of not much more than one-half of the original supply of yolk. The two members lie with their ventral surfaces in apposition, and they are united from the level of the yolk-stalk backwards to the tail. The union is thus one of Gemmill's Class VI (**2**, p. 21). The larger member (A), in Plate V, appears normally developed with respect to the head,



TEXT FIG. 7.—Gut of Double-monster of *Acanthias vulgaris* Risso.

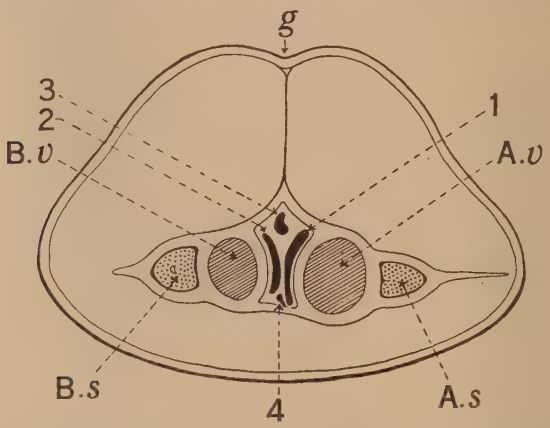
A. ST. Stomach of larger member A.
A. SP. Spleen of larger member A.
A. P. Pancreas of larger member A.
B. ST. Stomach of smaller member B.
I. Intestine.
R. Rectum.
Y. Opening of internal yolk-sac into intestine.

pectorals and two dorsal fins; the smaller member (B) also exhibits normal gills, pectorals and dorsals, while the head is typical save for malformed nostrils. The nostrils in (A) are quite normally separated laterally by an appreciable interval, whereas in (B) they are adjacent and lie in a shallow pear-shaped depression (Plate V (*n*)). The general pigmentation in both members is normal and most effectively indicates the identity of each. There is but one pair of pelvic fins (female in structure) situated on the left side as the monster is viewed with the larger member uppermost. On this same side a groove occupies the middle line from the anus backwards towards the tail. Still viewing the specimen with member (A) uppermost, it is seen that the body of the latter passes by a rather abrupt downward kink about 1 cm. behind the

second dorsal fin into an almost normal heterocercal tail. Tracing the smaller member (B) backwards it would seem to possess no tail, the body stopping short somewhat suddenly to fuse with that of (A) at the level of the kink. At the root of the single well-formed tail, however, there is a small fleshy lobe, (c) in Plate V, left, which may represent the tail of (B).

Digestive System.

The digestive organs of the specimen presented an interesting condition which is, perhaps, most conveniently described by starting from the single short rectum (Text-Fig. 7, R) and working forward. The rectum,



Text Fig. 8.—Hand-section of Double-monster across tail-end behind pelvics. Section cut in direction S—S indicated in Plate V.

A. v. centrum of A.
B. v. centrum of B.
g. external groove.
A. s. spinal cord of A.
B. s. spinal cord of B.
1, 2, 3, and 4. Blood-vessels in united hæmal arches.

passes into a median intestine (I) with a spiral valve of the usual character. Near its front end the intestine receives on either side a two-limbed stomach. The larger of these two stomachs (A. st.) can be traced to the œsophagus of the member A, and is supplied with a normal pancreas (A. p.) and spleen (A. sp.). The smaller stomach (B. st.) leads to the œsophagus of the member B, but, unlike its fellow, is without either pancreas or spleen. At the front end of the intestine there is communication (Fig. 7, Y) with the internal yolk-sac. The latter is a bi-lobed organ, communicating on the one hand, as stated, by a median opening to the intestine at Y, and, on the other, to the external yolk-sac by a single channel in the yolk-stalk. Both members of the monster have

a bi-lobed liver, but that of member B is much the smaller and very unequally lobed.

Hand Section across the Tail-region.

A hand section across the tail a short way behind the pelvic fins is shown diagrammatically in Text-Fig. 8. It is seen that members A and B retain their identity so far as the centrum and spinal cord are concerned, but that there is fusion of the hæmal arches, enclosing the blood-vessels of both. In the description of the external features of the specimen, reference was made to the small fleshy lobe (c) at the root of the single normal heterocercal tail, and the suggestion was made that (c) may represent the tail of B. Sections cut posterior to the one just described lend support to this view in that the identity of B can be traced backwards to (c).

The monster would thus appear to be the union of two embryos, of which one (A) is dominant. In a letter to *Nature* on May 25th, 1929, von Bonde and Marchand (**7**, p. 795) describe a somewhat similar union in *Squalus fernandinus*. Their specimen differs in having a more advanced duplication of the tail, while the nostrils of both members appear normal. The section across the tail region shown in their Figure 2 also differs from that represented in Text-Fig. 8 of this paper in several respects. In the section of *S. fernandinus* the blood-vessels are shown separated from the "lateral arch" facing the external groove by a thick line, presumably indicative of cartilage. But in *Acanthias vulgaris* a blood-vessel (Text-Fig. 8, 3) is seen to occupy this lateral arch, there being no cartilaginous cross-piece separating a hæmal arch from a "lateral arch." At the time of writing their letter von Bonde and Marchand had not dissected their specimen for an examination of the internal organs. It will be interesting to know, when they have done so, how far their specimen agrees with the present one in the characters of the alimentary canal.

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EXPLANATION OF PLATES.

PLATE I.

Sagittal Section of Head of Malformed Cod.

d. original left-half of lower jaw.
t. tongue lying in extension of mouth-cavity.
h. heart.

PLATE II.

Fan-tailed Pilchard.

Left: From X-ray photograph by Institute of Anatomy, University College, London.
Lower right: Tail of Fan-tailed Pilchard.
Upper right: Tail of normal Pilchard of approximately same length.

PLATE III.

Abnormal specimen of *Pleuronectes microcephalus* Donov. From X-ray photograph by Institute of Anatomy, University College, London.

PLATE IV.

6 A. Abnormal *Raia microcelluta* Mont.
6 B. Abnormal *Raia clavata* L.

PLATE V.

Double-monster of *Acanthias vulgaris* Risso. (Photo, C. H. Gill.)

A. Larger member.
B. Smaller member.
c. Rudimentary tail of B.
n. Abnormal nostrils of B.
y. Severed end of yolk-stalk.

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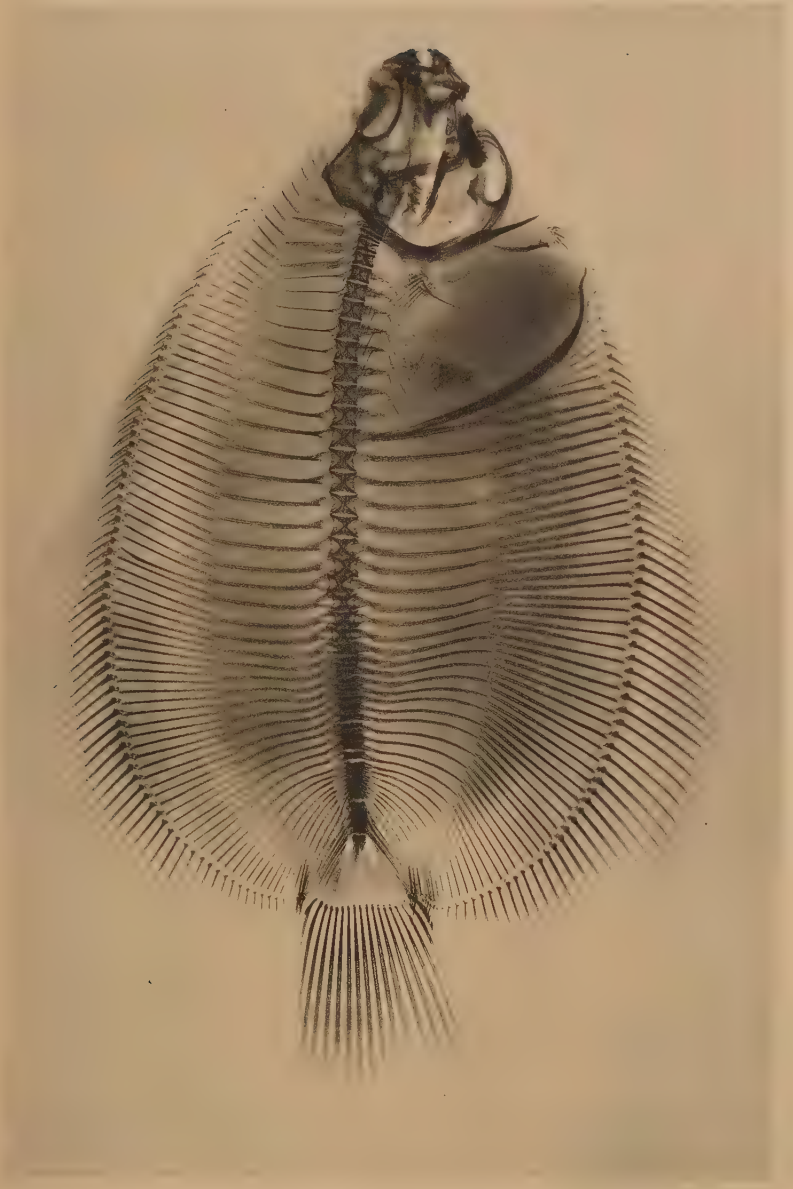

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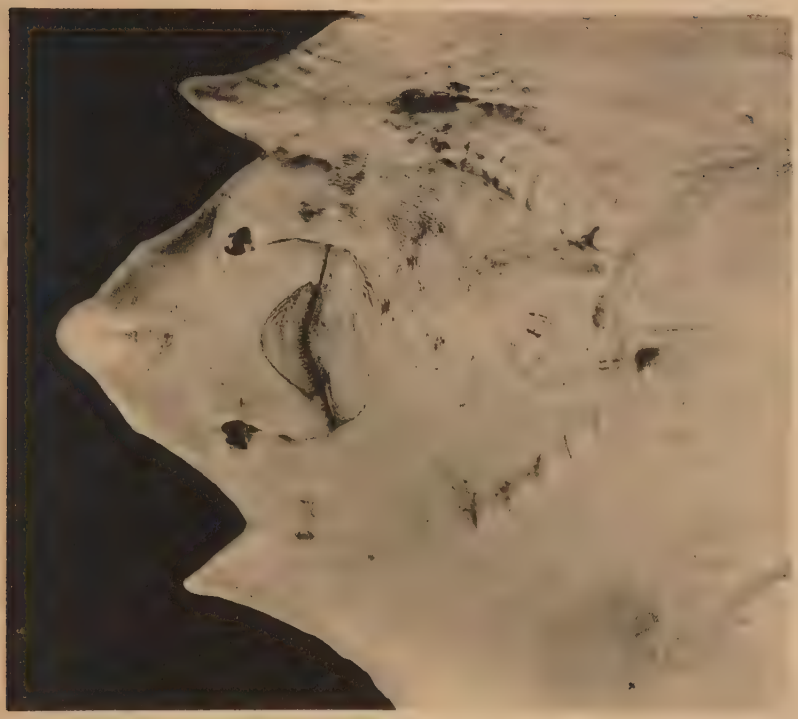

6B

6A

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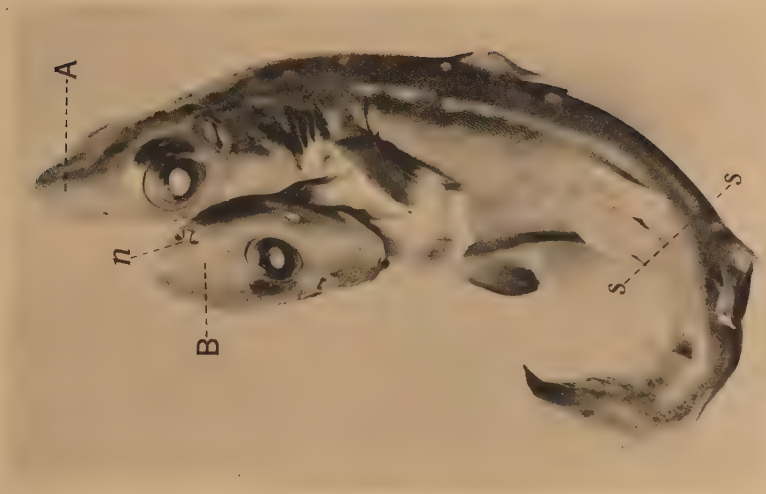


On the Hypothesis of Mitogenetic Radiation.

By

Dr. Nine Choucroun.

With 3 Figures in the Text, and Plates VI and VII.

I. Introduction.

In order to explain certain actions influencing at a distance the cellular development of living tissues, various authors have framed the hypothesis of a *mitogenetic radiation* emanating from cells in the act of division.

Gurwitsch (**1**) first of all pointed out the mitogenetic influence of cells of embryonic tissues, in the form of an emulsion or otherwise, on the root of the onion or on yeast cells. According to him, this action, which was exerted through sheets of quartz—but not of glass—is due to a radiation, or, to be more explicit, to an ultra-violet radiation of wave-length 2000 Ångström units, produced by the active material and capable of regular reflection.

Reiter and Gabor (**2**), who have since then carried out many more such experiments, also attribute the action to a mitogenetic radiation, the wave-length of which they have measured (according to them nearer to the visible) and can readily follow, so that, for example, they can separate the two rays reflected from the opposite faces of a sheet with parallel faces.

An analogous influence had been observed by M. Joseph Magrou and Mme. M. Magrou by exposing to *Bacterium tumefaciens*, through a sheet of quartz, a root or a stem of an onion, and later sea-urchin eggs. In the first case they verified the phenomena observed by Gurwitsch, without however affirming the existence of a radiation. In the second case they obtained aberrant larvæ, plutei opaque and with short arms or armless, instead of plutei transparent and elongated as are the normal larvæ. Histological examination seemed to indicate that these differences in appearance were related to differences in structure; in the irradiated larvæ the mesenchymatous cells were more numerous than in the controls. This case also might be explained on the theory of a mitogenetic action.

On the other hand, it did not seem that this action could have been due to a vapour carried by the air, for the controls, placed in the immediate neighbourhood of the lots exposed to radiation, gave a perfectly normal

development. M. et Mme. Magrou were of the opinion that this action too, just as in the case of the onion root, could be attributed to a hypothetical radiation emanating from the bacteria.

But the action producing malformation was not always evident in the exposed lots, which indeed were not very numerous, and sometimes even the effect was not produced in a single one.

Unsuspected causes of lack of symmetry might conceivably have been at play either in themselves to provoke the observed phenomena or to prevent the action exercised by *B. tumefaciens* from becoming manifest.

It became a necessity, therefore, to establish with certainty the reality of an action causally connected with proximity to the culture. And for that an effort had to be made to realise, in the steps we took, the best possible conditions of symmetry and homogeneity for all the batches, controls and exposed.

II. Reality of the Phenomena.

To prove the reality of the phenomena was the task for us, M. Magrou, Mme. Magrou and myself, at the Marine Laboratory of the Institut Océanographique de Monaco (**3**).

This time we worked with a larger number of batches, about 24, namely, 12 controls and 12 exposed, selected at random. The controls were, for the greater part, placed on a table among the exposed.

Eggs of *Paracentrotus lividus*, from the very same animal and laid at the same time, were all fertilised in the same vessel by the same supply of sperm. Immediately after fertilisation the eggs were divided in a manner as similar as possible among the small quartz receptacles ; these were 3·5 cm. in diameter with optically-worked bottoms. Certain of these receptacles were placed on vessels of the same diameter containing a thick suspension of *B. tumefaciens* in nutritive broth, the suspension coming from a single supply of emulsion.

All the lots, control and exposed, were placed in identical conditions as regards illumination, air supply and temperature, in an incubator kept near 22° C., which temperature is favourable for the good development of the culture and is borne very well by the eggs used.

Under these conditions we verified the production of abnormalities observed in the earlier work, and the results obtained, based on a number of experiments, allow us *to conclude that there is a definite causal connection between the existence of an action and the presence of the culture.* But, as before, a certain proportion of the exposed lots (this time always about the same in the successive trials, thanks to the greater number of the receptacles) escaped all action.

It was natural to expect, on account of the degree of homogeneity

realised, that in each lot a certain proportion of the eggs were more resistant than the others to the injurious action observed, giving rise therefore to larvæ normal in every respect. But it was difficult to admit that entire lots, made up of the same larvæ as the others, could completely escape the action if some cause of lack of symmetry were not in existence.

One might incriminate the culture and ask whether, once it had been subdivided, it retained the same activity throughout. As a fact, in certain vessels, the culture settled out, whereas in others it remained in an emulsion. Sometimes the absence of action coincided with this precipitation of the culture and sometimes it did not do so. In any case, this possible cause of lack of symmetry would disappear if one were to submit all the lots to the action of one single culture, and if in addition this culture were to revolve beneath the batches of sea-urchin larvæ kept at rest. This is what I proposed and carried out as follows :—

III. Realisation of a Homogeneous "Irradiation."

An annular vessel, A, of pyrex glass, about 30 cm. external and 25 cm. internal diameter, intended to contain the bacterial suspension, rotates around an axis O (Fig. 1) below another annular vessel, B, which covers

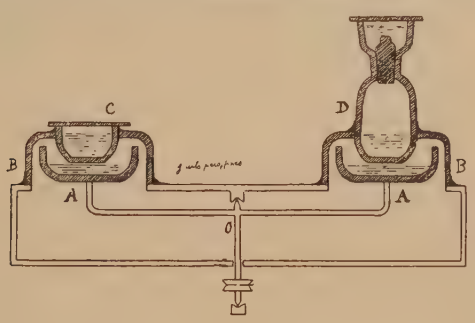


Fig. 1.

it. Vessel B is pierced by circular holes in which are affixed, by joints of paraffin wax, the quartz receptacles, C, which contain the lots of sea-urchin larvæ.

The vessel A is carried by an annular plate of metal which revolves around an axis, O, with a uniform and slow movement, about one turn in eight minutes. The upper vessel rests partly upon the edge of the apparatus, partly upon a central fixed plate, the mechanical disposition of the parts permitting of the complete separation, by means of paraffin wax joints, of the air in contact with the culture from that in contact with the sea-urchin eggs.

An exactly similar control apparatus functions at the same time, the only difference being that in it the vessel A contains no culture (Fig. 2).

Under these conditions of homogeneous "irradiation" one might expect to observe an absolute identity in the action. Now, in the experiments carried out at the Marine Laboratory of Banyuls, we indeed observed progress in this respect; but in certain experiments, irregularities existed (exposed lots remaining normal) which it became difficult to explain on the hypothesis of a radiation once homogeneity of irradiation had been realised.

On the other hand, the photographic plate substituted for the sea-urchin eggs never revealed the supposed radiation, in spite of very long exposures (48 hours). Stated more precisely, with the conditions

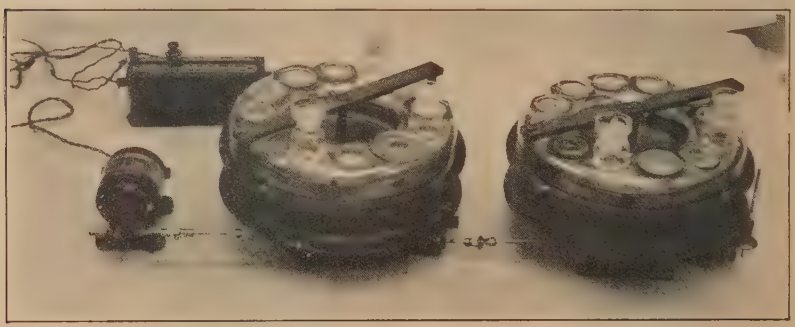


Fig. 2.

under which the phenomena became manifest in the eggs, the plate, even when sensitised to the extreme ultra-violet, received absolutely no impression. I must say that for many physicists this negative result sufficed to render doubtful the existence, in this case, of a mitogenetic radiation.

I then applied myself, in a new piece of work carried out this time at the Marine Laboratory at Plymouth, to test this hypothesis of radiation.

IV. It is not a Radiation which Acts.

To investigate the above proposition, certain of the quartz capsules were replaced by quartz flasks, D, with ground stoppers, the base of which was intentionally constituted of capsules already used in the preceding experiments, and of which the stoppers were surrounded by a trough filled with frequent changes of sea-water (Fig. 1).

Each experiment consisted of twelve lots exposed (8 open capsules and

4 stoppered flasks) carried by the apparatus containing *B. tumefaciens* and 12 controls (10 open capsules and 2 stoppered flasks) carried by the other apparatus. According to the radiation hypothesis, the action should be shown in the same manner in the stoppered flasks and in the capsules. One should observe in these flasks an abnormal development simultaneously with a normal development in the control flasks of the blank apparatus.

Now, never did I observe any abnormality in the exposed stoppered flasks. The same culture of *B. tumefaciens* which exercised a strong action on the eggs in the capsules produced no effect on the eggs in the stoppered flasks ; the water-seal sufficed to stop all action. In three of the experiments, all the capsules exposed gave abnormal developments, whilst the four stoppered flasks gave normal developments as well as the twelve receptacles (capsules or flasks) of the blank apparatus.

In the photograph reproduced here by way of example, it may be seen that in the lots exposed in stoppered flasks the development is normal and comparable to what it is in the control vessels, whereas the anomalies are striking in the lots of the exposed capsules (Plate VI).

No longer, then, can one explain the observed action at a distance by a special radiation which exerts its effect through comparable media and would clearly reach equally well both the batches of eggs in the capsules and in the stoppered flasks.

More generally, and this is obvious, it is not possible to explain the observed phenomena by any action exerted *through* quartz vessels, for then it could not possibly suffice to close the flasks to stop all action.

V. Detailed Description of an Experiment.

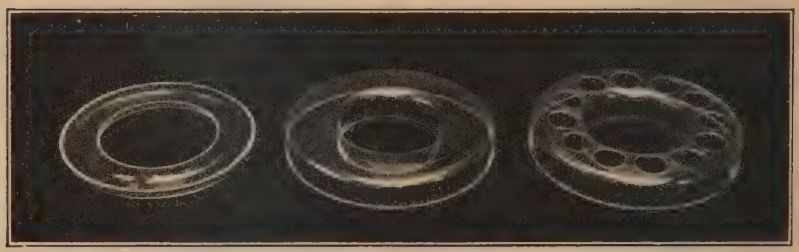
Before speaking of the observations, that I have been able to carry out, in the light of a different interpretation, I should like to describe in detail one experiment, laying emphasis upon the precautions that I had to take to realise, in the steps we took, the greatest possible similarity between the exposed lots and the controls.

All the *receptacles* were washed for a long time with concentrated hydrochloric acid, then with water, then with alcohol, then with distilled water, after which they were sterilised. The same precautions were taken for the vessel, A, before containing the culture, for the perforated covers, B, also for the non-perforated covers, B_1, which were to protect the vessel A during filling or transfer to the mechanical apparatus (Fig. 3). The receptacles, capsules and flasks, were divided, *haphazard*, between the two perforated covers resting on sterilised paper. All the joints were sealed with paraffin wax (M.P. 50° C.), then with soft paraffin (M.P. 38° C.) to fill up possible cracks.

The sea-urchin *eggs*, of the species *Echinus miliaris*, coming from one oviposition were washed for a long while with fresh sea-water, then fertilised all at once by the same sperm suspension ; they were then separated by decantation from the water in which they had been fertilised and finally suspended in a volume of sea-water sufficient for the eggs to develop well and withal small enough for their concentration in each receptacle to render observation easy. They were then divided uniformly, by the aid of a sterilised glass spoon, between the various receptacles, ladling out alternately the lots for the apparatus with *B. tumefaciens* and those for the control.

Once more must I insist upon the necessity for respecting the conditions of symmetry up to a point which may appear puerile.

The *Bacterium tumefaciens* was cultivated on agar (at a temperature of 38° C.) in large Roux flasks. It was then emulsified in ordinary



A. B_1. B.

Fig. 3.

broth Ph. 7·4 and the emulsion from each flask was poured with aseptic precautions into the vessel A furnished with its cover, B_1. With three Roux flasks a thickness of about 5 mm. emulsion was obtained. This vessel A and the empty control vessel, both furnished with their protective covers, B_1, were carried on the rotating plates of the apparatus. For these covers, B_1, were substituted the perforated covers B, carrying the vessels the bases of which were then within a few millimetres (about eight) of the free surface of the culture. The inner and outer edges of these covers were paraffined with a brush and the motors turning the plates bearing the vessels A were started. The experiment was allowed to proceed till the characteristic " pluteus " stage was reached, renewing, about every four hours, the sea-water contained in the troughs of the stoppered flasks so as to maintain a fresh water-seal, free from impurities.

The first experiment was carried out at a temperature of 17° C., the apparatus being enclosed in a water-oven regulated for this temperature. But the renewing of the water of the water-seal was difficult inside the oven. Accordingly in the following experiments the apparatus were

simply placed upon the laboratory table. If necessary the general temperature was lowered so as not to exceed 20° C. Moreover, some additional control lots were placed right up against the apparatus containing *B. tumefaciens* as a test that, as was already known, the abnormalities were not produced by the very small temperature differences which might exist between the atmospheres of each apparatus.

As regards *observation*, each lot was examined in its position by the aid of a binocular glass which allowed one to have in the field, and in its depth, a sufficiently great number of larvæ for an estimate to be made of the relative proportion of the normal and abnormal larvæ in each lot.

All the lots having been examined and the observations recorded; *photographs* of the experiment were made, working in such a manner as to be entirely ignorant as to whether a control or an exposed lot was being taken. From each lot, after shaking, a small quantity of the emulsion was taken and placed in a numbered test-tube. To each test-tube were added a few drops of 1% chloral to stop the movement of the larvæ which then became aggregated at the bottom of the tube. Using a very finely drawn-out pipette a small quantity of liquid containing larvæ was removed from each tube and placed upon a microscope slide. The number of the corresponding lot was written on a label on the slide and *hidden*.

Then from among all the possible fields a choice was made of one which gave the best representation of the general aspect of the preparation.

It is only thanks to these precautions, of which some might seem puerile, that the photographs taken represent *objectively* the general aspect of the lots examined. This was indispensable to allow those who only saw the photographs to form an independent opinion.

VI. Attempt at Explanation.

Let us now return to the phenomenon itself.

If it seems no longer possible to explain the anomalies observed by a radiation, nor in a more general form by any action involving "passage through," it is equally impossible to doubt the existence of an action clearly established and confirmed by so many new experiments.

It seems difficult then to avoid thinking that something material, given off by the culture, reaches—in spite of the paraffin wax joints—the medium in which the eggs are developing, *travelling round* the wall of the receptacles.

It is fairly frequently observed that a bubble develops at the base of the

exposed capsules. One may then raise the question as to whether a *film, monomolecular perhaps*, may not start from these, spread over the wall, insinuate itself between this wall and the paraffin joint, and introduce into the medium, in which the eggs are developing, very minute amounts of a substance which suffice however and this is very remarkable—to exercise on the eggs an exceedingly injurious effect.

Concerning this I have made the following observations: in two experiments, certain quartz capsules contained only sea-water both on the apparatus with *B. tumefaciens* and on the blank apparatus. On each perforated lid there were five capsules with eggs, five with sea-water only and two stoppered flasks with eggs.

After having ascertained that the bacterial culture had acted on the lots of sea-urchins exposed in the capsules (never any action on those in the flasks) I withdrew the active culture; then I placed sea-urchin eggs (coming from a newly fertilised batch) in the sea-water which had been exposed, and in the unexposed sea-water of the blank apparatus.

Development was *very abnormal* in the receptacles which had been exposed to *B. tumefaciens* and normal in the controls. In one of the two experiments, stopped at the gastrula stage so as to be able to photograph certain peculiarities, the injurious action had been particularly intense. In one of the lots (Plate VII) the eggs had not even divided; in the others many eggs had not reached the blastula stage. In the majority of the lots advanced cytolysis could be observed even before the gastrula stage had been reached. This would seem to indicate that the active impurity is specially active during the first stages of development. According to our hypothesis of the transport of something material, however small it be, by a monomolecular film, it is understood that exposed sea-water must be, for the eggs introduced into it, a different medium from that in which the eggs are when themselves directly exposed to the culture. In this case, the active impurity, nothing at the start, goes on increasing during the experiment; whereas in the former case there has been, right from the beginning, a relatively great concentration.

Thus, exposed sea-water has been modified thanks to proximity to the culture, and this in spite of precautions which seemed adequate to guard against all material action.

The objection has been raised that the sea-water itself might very well have been modified by a hypothetical radiation. But then it would be impossible to understand why this modification had not been produced in the sea-water of the stoppered flasks containing eggs, necessitating an abnormal development for the eggs.

VII. SUMMARY.

1. The observed " action at a distance " undoubtedly exists.

2. This action cannot be ascribed to a radiation, nor more generally to an action " through the wall " of the receptacles.

3. This action must then be exercised " across the surface " of the wall of the receptacles by a mechanism which remains to be explained. This seems, however, to be of great interest in view of the apparently very small mass of the substance concerned in the action.

4. The action of something material is rendered probable from the fact that the eggs develop very abnormally in sea-water previously exposed to the active culture.

5. Although these experiments have only been carried out upon sea-urchin eggs, their conclusions may be valid in the case of the very numerous experiments concerning mitogenetic radiation.

In the first experiments it was thought that the sea-urchin eggs had been shielded from the action of any material emanating from the culture. An additional precaution (stoppered flasks) sufficed, however, to bring it about that the better isolated eggs remained insensible to the action at a distance which continued, however, to be exercised upon the other receptacles, which were less efficiently protected.

Experiment has shown that a water-seal sufficed to protect the eggs against all action producing deformity. If in this case abnormalities had again been found it would have been necessary to substitute sealed flasks for stoppered flasks. Only then, and if the abnormalities had again been found in sealed flasks exposed, but not in sealed control flasks, would it have been possible to conclude that the existence had been established of an action at a distance by means of a radiation, or more generally, an action exercised *through* quartz.

As far as the actions not studied here are concerned the conclusion must certainly be drawn that the existence of a radiation has not been proved.

It would at least be necessary, in order to carry conviction in this respect, that *rigorous* precautions should be taken to hinder *absolutely* all transport of material between the activating substance and that used as a test in the observations.

It has been possible to carry out these researches thanks to a grant from the Institut de Biologie physico-chimique, founded by M. Edmond de Rothschild.

On the other hand, I must specially thank Dr. E. J. Allen for the hospitality afforded to me in his laboratory. It has been very valuable to me, in the course of my work, to profit by the resources of the laboratory and above all from the friendly atmosphere created by Dr. Allen and the numerous research workers surrounding him. I should like to thank them all.

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EXPLANATION OF PLATES.

PLATE VI.

Experiment III. Shows normal development of sea-urchin larvæ in stoppered flasks and control vessels, and abnormal development in exposed quartz capsules.

PLATE VII.

Experiment VIII. Shows abnormal development of sea-urchin eggs which had been placed in "exposed" sea-water, and normal development of controls.

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The Absorption of Fats and Lipoids in the Plaice (*P. platessa* L.).

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With 11 Figures in the Text.

CONTENTS.

	PAGE
Introduction	75
Historical	75
Brief Statement on the Technique of Fat Staining	77
Material and Methods	78
Notes on the Morphology and Histology of the Alimentary Tract	79
Protocols (1) to (7)	80
Absorption of Fats and Lipoids in the Stomach	90
Absorption in the Duodenum, Pyloric cæca, Intestine and Rectum	91
Summary and Conclusions	99
Literature	100

Introduction.

In a previous paper, an account was given of the histological structure of the alimentary tract of the plaice, which account was submitted as the basis of a series of studies on secretion and on the digestion and absorption of food substances in this fish. The aim of the work which is to be described presently is to show histologically how fats and lipoids are transported through the mucosa of the various regions of the alimentary tract and to indicate which regions are most concerned with the absorption of these substances. The possible function of lipoids as intermediary products in the resynthesis of true fat within the epithelial cells is considered and discussed briefly. The rôle of mitochondria and golgi elements in fat absorption has not been determined, presenting as it does a distinct and separate problem, but useful fat pictures have been obtained, as have also data on the question of absorption in fishes.

Historical.

It appears that Weber (1847) was one of the first workers to demonstrate histologically the absorption of fat. He observed that during

fat digestion and absorption the cells of the duodenal villi become swollen and opaque, or filled with transparent, oily fluid. Kölliker (1857) demonstrated the absorption of fat by the stomach of the mouse, cat and dog, and observed that fat is always present in the gastric mucosa in variable quantities from the second day after birth onwards. Krehl (1890) showed that fat is taken up in solution and not in globular form and that it is re-formed into droplets within the cells of intestinal ephithelia. His observations and conclusions received support from Pflüger (1900). Schilling (1901) observed fat in the gastric epithelium of the calf after a milk meal and noted the occurrence of fat droplets in the connective tissue beneath this epithelium and also in the lymphatic glands. In 1908, van Herwerden published comprehensive studies on gastric digestion in fishes, chiefly sharks but also teleosts. He fed fish artificially with oil or egg-yolk emulsions and observed that a number of hours later, fat drops were to be found in great numbers in the epithelia and sub-mucosæ, which tissues he noted as being free from fat during starvation. His work appears to be the first of its kind carried out upon fishes. Greene (1912) demonstrated histologically the absorption of fats by the stomach of the salmon, later (1913) extending his observations to include all regions of the alimentary tract, finding that the primary function of the pyloric cæca is that of fat absorption.

A host of workers attacked the problem from the chemical point of view, and it is interesting if not entirely relevant to consider briefly the general trend of this work. Marcet (1858) and Cash (1880) were concerned with the lipolytic properties of mucosa extracts, noting increased fatty-acid content of the stomach during the digestion of a fat meal. Interest centred chiefly on the questions of the reversibility of lipolytic activity and the resynthesis of fats, and the researches of Brucke, Rochford, Monk, Moore and Rockwood, Ewald, Hamburger, Cunningham and others led to the classical investigations of Kastle and Lœvenhart (1900). From these investigations it is held that fats are first split up into fatty-acids and glycerol, which products pass through the borders of alimentary epithelia to be resynthesised to form fat again within the epithelium cells as a result of the reversible nature of lipolytic activity. Moore (1903) and Frank and Ritter (1906) could find no evidence of the occurrence of resynthesis " in vitro " and the theory does not receive universal support. Bradley (1913) attempted to discover some correlation between the quantities of storage fat and the concentration of lipase in certain tissues of a variety of marine animals. Instead of correlation, he found that some of the tissues richest in fat are poorest in lipase and vice versa, and concludes that a comparison of fat and lipase in animal tissues gives no positive evidence in support of the theory of enzyme synthesis.

It is generally accepted, however, that the cleavage products of fats are

passed through the clear margins of epithelia to be resynthesised in some way to form fats again within the cells. Mayer, Rathery and Schæffer (1914) suggested that mitochondria possibly supply phospholipid which serves as an intermediate stage in resynthesis, while Cramer and Ludford (1925) show that the golgi apparatus may be the cell structure mainly concerned, and that mitochondria do not appear to take an active part in the process of fat absorption. Sinclair (1929), adopting a chemical point of view, finds that during the absorption of fat there is a pronounced change in the composition but no change in the amount of phospholipid fatty-acids of the intestinal mucosa and of the liver. He suggests that absorbed fatty-acids are transformed into phospholipids in the cells of the intestinal mucosa, as an essential step in the resynthesis of fat.

Brief Statement on the Technique of Fat Staining.

Albrecht applied the term "liposome" to small refractive droplets observed in freshly mounted sections of liver and other tissues. These droplets appear more numerous and more sharply defined if the sections are cleared in potassium hydroxide solution before mounting. But they are not proven to be entirely lipoid in character, and Bell (1910) uses the term in a more restricted sense to denote such of these drops as may also be stained with Herxheimer's Scarlet Red (2·0 gm. NaOH added to 100 c.c. of 70 per cent alcohol with subsequent addition of Scarlet Red to saturation). Osmic acid and simple alcoholic solutions of Scarlet Red and Sudan will stain many of the liposomes but do not invariably give positive results. Altmann (1894) demonstrated the reduction of osmic acid by oleic acid and triolein and showed that no reduction takes place with tripalmitin, tristearin or their acids. Starke (1895) and others, maintain that osmic acid will blacken all fat if sections are kept for some time in alcohol after the osmic treatment. He concluded that palmitin and stearin do reduce osmic acid, and Handwerck (1898) agreed with him, but maintained that at least a trace of olein was essential if reduction was to occur. Heidenhain (1907) rejected the osmic-alcohol treatment, which blackens bodies that are not true fat and does not affect others which undoubtedly are fat. Mann showed that osmic acid may be reduced and hydrated by alcohol into $OS(OH_4)$ and rejected the osmic-alcohol treatment. Bell (1910) found that "on the whole osmic acid is a useful reagent for the study of liposomes. It often gives a brown colour to droplets not stained at all by the simple alcoholic solutions of Scarlet Red and Sudan." Gatenby (1919) holds that the blackening effect of osmic acid can take place in the presence of a chrome compound, e.g. chromic acid, and that fresh palmitin and stearin are blackened by osmic acid after treatment with formalin. In the *Microtomists' Vade-Mecum* (1928)

we are told that "the true fats and the lipoids are all blackened by osmic acid," and that the various groups differ in the readiness with which they are blackened as well as in the depth of blackening produced. True fats blacken most readily and deeply, while substances such as cholesterol are not readily blackened. Treatment with bichromate solutions accentuates these properties and prevents substances showing only weak reducing powers from reducing osmic acid, so that after such treatment only true fats and fatty-acid, cholesterin mixtures are blackened. In order to examine lipoids, Ciaccio fixed tissues in a formalin-bichromate acetic mixture which he believed fixes lecithin so that it is not removed by the ordinary fat solvents. Bell maintained that it is not proven that the droplets so preserved consist of lecithin. Ciaccio (1921) preserved fatty acids of the saturated series by fixing small pieces of tissue in a saturated solution of zinc acetate, staining sections with Sudan or Scarlet Red.

Material and Methods.

The fish used in the following experiments were trawled in Cawsand Bay or in the nearby vicinity by s.s. *Salpa* of the Marine Biological Association. As soon as possible after trawling they were placed under circulation and later transferred to fish-boxes in the pond at Pier Cellars, boxes such as are at present being used in connection with work on Growth and Maintenance in the Plaice, and were allowed to become settled. Individuals showing signs of any ill-effect due to trawling were removed at once and the remainder were fed regularly on a diet of *Mytilus edulis* fragments. All the fish used took food avidly and were apparently perfectly sound, and the physical conditions under which they were kept approximated closely to the natural ones, the fish living in boxes in the sea.

Primarily, a number of fish were fasted for several days, after which they were killed by a blow on the head which pithed the brain and the tissues of various regions of the alimentary tract were appropriately fixed to serve as controls. Other fishes were fasted for two or three days, fed in various ways and killed the requisite number of hours afterwards in order to preserve tissues in various stages of absorption. Several lines of treatment were adopted. In some cases, a single meal of Mytilus or Nereis was supplied, the time at which food was taken being noted. In other cases, a second meal of similar food was given at various times after the first, but usually about twenty-four hours later. Because of the great avidity shown by the fish for food, the stomach was invariably filled during a few minutes following the presentation of food, while the fish were still under observation. Thus the times could be recorded with great accuracy. Several fishes were given olive oil or olive oil emulsion

meals by injection through the mouth both during the "empty" condition of the gut and also following upon a meal. And, finally, a number of fishes were fed for several consecutive days upon Mytilus fragments liberally mixed with olive oil. Freshly trawled fish were used in several instances.

Several methods of fixation were employed. Initially frozen sections were prepared of material fixed in 10% formalin, which sections were stained with Sudan or osmic acid. But this method was discontinued in favour of more applicable ones. Some material was fixed in equal parts of Müller's fluid and 1% osmic acid, but only in a few instances could this method be regarded as successful. Comparison was afforded with tissues fixed by Kolatschev's slightly modified Champy fluid, and this latter fixative was largely used subsequently with great success. Tissues were kept for 24 hours in fluid consisting of 4 parts of 1% chromic acid, 4 parts of 3% potassium dichromate, and 2 parts of 2% osmic acid. They were washed for several hours in running water and treated for 3 days with 1% osmic acid. Sections were mounted in xylol balsam, Apathy's gum syrup and glycerine, those in balsam lasting well.

Materials corresponding to those fixed in Champy fluid were also fixed by Ciaccio's method (Schmorl, 1925) in a mixture consisting of 80 c.c. of 5% potassium bichromate, 20 c.c. of formaldehyde, and 5 c.c. of glacial acetic acid. This was followed by treatment for 5–8 days in a 3% solution of potassium dichromate, after which the tissues were washed for 24 hours in running water and passed through alcohols and xylol to wax in the usual manner. This technique is claimed to preserve lipoids, although true fats are removed in the alcohols and xylol. A potent solution of Sudan was obtained by dissolving the dye in boiling 85% alcohol, as Fischer suggested for fats. To 95 c.c. of this solution, 5 c.c. of acetone were added and the stain applied to the sections for an hour or more at a temperature of about 30° C. The sections were then differentiated and counterstained faintly with iron hæmatoxylin. This technique also gave good results. The mounting medium employed was Apathy's gum syrup.

Notes on the Morphology and Histology of the Alimentary Tract.

The alimentary tract of the plaice is differentiated into regions, of which the important ones from the point of view of absorption are stomach, duodenum, intestine and rectum. The stomach comprises about $\frac{1}{6}$th of the entire length of the tract and is separated from more posterior regions by the pyloric valve. The post-pyloric intestine, which takes up approximately half the whole length of the tract, is for convenience

regarded as consisting of two equally long regions, the duodenum and intestine. Four very small pyloric cæca open into the duodenum immediately posterior to the pylorus. The terminal portion of the tract or rectum is separated from the intestine by a second valve, the intestino-rectal valve.

Histologically, the tract consists of a serosa, outer longitudinal and inner circular muscle bands, an areolar tissue layer and a mucous membrane. The mucous membrane of the stomach is very thick, being formed into numerous simple, or slightly branched, tubular glands consisting of three types of cell, (1) columnar cells forming a superficial epithelium, which is continued into the crypts marking the openings of the glands, (2) cubical, mucus-producing cells, which form the neck of each gland and (3) granular cells which build up the basal portions of the gland tubules. Processes of the areolar tissue layer pass between adjacent gland tubules. In the duodenum, with its pyloric cæca, intestine and rectum, the mucous membrane consists of a simple epithelium of extremely tall and slender columnar cells interspersed with goblet cells. It is gently corrugated into a large number of folds which simulate, but are not, villi. The areolar tissue layer follows the mucosal folds, passing into the tips of even the most slender of these. The degree of mucosal folding is much greater in the pyloric cæca than in other parts of the post-pyloric intestine.

Protocols.

(1) *Plaice, 20·0 cm. in length, killed 30 hours after taking a single meal of Mytilus fragments. Tissues fixed by modified Champy and Ciaccio methods.*

Stomach. The superficial epithelium shows a moderately heavy loading with fat, in osmicated material, although some folds are relatively fat free. Fat globules are relatively scarce in the cells of the crypts and altogether absent from the mucus-producing cells forming the necks of the glands (Fig. 1). The cells of the basal portions of the gland tubules each contain several fat globules ranging in numbers from one or two to about ten (Fig. 1). The areolar tissue layer is devoid of fat. In the cells of the superficial epithelium the fat globules are distributed on each side of the nucleus, but are larger and more numerous on the outer aspect of it. The outer $\frac{1}{5}$th part of each cell is devoid of fat (the clear cell margin takes up rather less than half of this). The next deeper $\frac{1}{5}$th part is densely crowded with moderately large globules, below which comes the region of the nucleus, where there are no globules. Smaller globules occur below the nucleus, extending to within $\frac{1}{5}$th part of the depth of the cell from the base (Fig. 2).

In chromed material, fine, Sudan-stained lipoid granules are distributed

somewhat similarly to osmicated fat. The outer halves of the cells are crowded but the outer $\frac{1}{5}$th is clear. In some parts of the epithelium, granules occur in groups below the nucleus, but these are not as common as are the fat globules.

Duodenum. Fine Sudan-stained lipoid granules are crowded in the

FIG. 1.—Vertical section of the gastric epithelium of the plaice referred to in Protocol (1). Unstained preparation of Nassanow—Champy-Kull material. Showing the loading of the superficial epithelium and the basal cells of the gland tubules with fat, and also the absence of fat from the mucus-producing cells forming the necks of the gland tubules. × 460.

outer halves of the cells at the tips of the mucosal folds. In osmicated material, corresponding cells show crowds of minute, brown-stained, and in some cases blackened, particles. The cells of the mucosal troughs are blank.

In all other regions of the alimentary tract fat globules are absent.

(2) *Plaice, 17·8 cm. in length, killed 50 hours after taking a single meal of Mytilus fragments. Fixation as before.*

Stomach.—The loading of the superficial epithelium with osmicable fat globules is greater than that after 30 hours' digestion (Protocol 1).

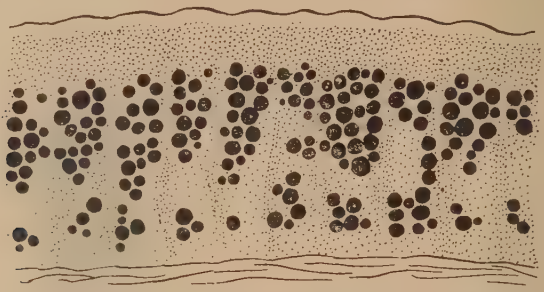

Fig. 2.—Vertical section of the superficial epithelium of the stomach of the fish referred to in Protocol (1). Unstained preparation. Tissue fixed by modified Champy-Kull. The sizes and distribution of the fat globules have been rendered as accurately as possible. ×1300.

Fig. 3.—Vertical section of the gastric mucosa of the plaice referred to in Protocol (2). Unstained preparation. C.–K. fixation. Showing a heavy loading of the superficial epithelium with fat except in the parts lining the crypts, where fat is relatively sparsely distributed. ×780.

The outer $\frac{1}{6}$th of each cell is clear, while in the body of each cell large droplets are arranged in two or three longitudinal rows lying so close as almost to touch each other (Fig. 3). In some folds, however, there is but little fat, as before. Fat is also relatively scarce in the cells lining the crypts (Fig. 3), whilst in the mucus-producing cells of the neck and the basal cells of the tubules fat is absent. Nor does fat occur in the areolar

tissue layer. In the gastric epithelium adjacent to the pyloric sphincter, where gastric glands do not occur, there is a considerable loading with fat droplets, which is not as heavy as in the glandular part of the stomach.

In chromed material, minute Sudan-stained lipoid granules are to be found crowded in the cells of the superficial epithelium. They occur in all parts of the cells but appear to be more numerous and more densely aggregated in the basal parts. They are much smaller than the droplets observed in osmicated material. The mucus-producing cells and basal cells of the tubules do not show such granules, although the latter cells

FIG. 4.—Vertical section of the intestinal epithelium of the plaice referred to in Protocol (2). Unstained preparation. C.-K. fixation. Showing the first stage apparent during fat absorption. Small droplets appear about $\frac{1}{3}$rd of the depth of the epithelium from the outer boundary. ×1100.

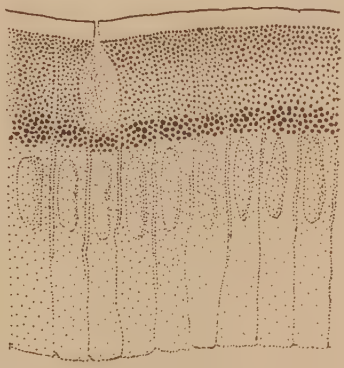

FIG. 5.—Vertical section of the intestinal epithelium of the plaice referred to in Protocol (2). The stage of fat absorption shown is slightly later than that shown in Figure 4. The droplets noted in Figure 4 are here enlarged and brown-stained and smaller droplets occur between these and the clear, cell margin. Unstained preparation. Fixation by C.-K. ×1100.

show dense aggregations of greyish stained granules in the half of the cells bordering the gland duct.

Pyloric cæca and Duodenum.—In osmicated material, cells near the tips of the mucosal folds show moderately dense clusters of extremely minute granules few of which are completely blackened. By far the greater part of the mucosa is devoid of fat, but clusters of cells are found to be partly filled with fairly large globules. These clusters occur in both duodenum and cæca but rarely.

Intestine.—Fat droplets are far more numerous than in the duodenal epithelium. They appear in the tips and limbs of almost every fold. In most folds they occur as extremely minute blackened bodies occupying a fixed position within the cells about $\frac{1}{3}$rd of the depth of the cells from the

clear margin (Fig. 4), so that under low powers of the microscope a thin, dark line follows the folds of the epithelium. But in some folds or portions of folds, larger droplets occupy this fixed position, the cytoplasm between these and the clear cell margin being densely crowded with smaller droplets (Fig. 5). In a few cells, small globules have been formed.

Minute, Sudan-stained granules are abundant in Ciaccio fixed material. These completely fill the outer halves of the epithelial cells, extending close up to the clear cell borders (Fig. 6). Undoubtedly, many of these granules appear brownish in colour in Champy fixed material. But there is no size differentiation in any parts of the epithelial folds and the granules are more numerous than the osmicated droplets.

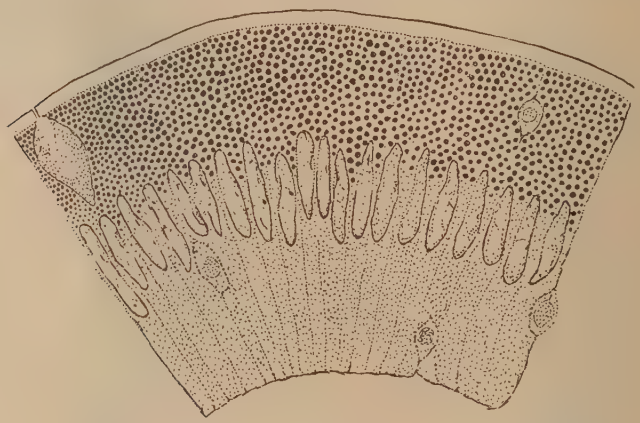

Fig. 6.—Vertical section of a portion of the intestinal epithelium of the plaice referred to in Protocol (2). From the limb of a mucosal fold. Fixation of tissue by modified Ciaccio. Stained with Sudan and iron hæmatoxylin. Showing the outer halves of the cells crowded with lipoid granules. $\times 1100$.

(3) *Plaice, 24·4 cm. in length; given a mussel meal, interval of* $26\frac{3}{4}$ *hours, given a second mussel meal and killed 19 hours later. Fixation as before.*

Stomach.—There is little or no osmicated fat in the superficial epithelium, except in the region of the pyloric sphincter, where the cells contain numerous small globules chiefly occupying a basal position.

In Ciaccio fixed material there are no Sudan-stained lipoid granules.

Duodenum and Pyloric cæca.—The epithelial cells are very heavily loaded with osmicated fat, especially in the tips and limbs of the mucosal folds, where the outer halves of the cells are rendered almost opaque with fairly large globules (Fig. 7). The inner halves of the cells contain fewer and smaller globules although much fat is present. In the heavily

loaded cells only the thin, clear cell margins are without globules. Stretches of epithelium, forming the limbs or troughs of the mucosal folds, do not contain fat, so that a single fold may carry much fat and yet possess cells without it. (Fig. 7 shows how the fat content of adjacent cells varies.) In the areolar tissue layer very small clusters of extremely minute droplets occur here and there, but generally, the layer does not contain osmicated fat.

In Ciaccio fixed material, minute, Sudan-stained lipoid granules occur in all parts of the mucosa, i.e. in the tips, limbs and troughs of the folds. The granules are very numerous, crowding the outer halves of the cells. In some cases the outer $\frac{1}{3}$th part of the cell is relatively clear of granules,

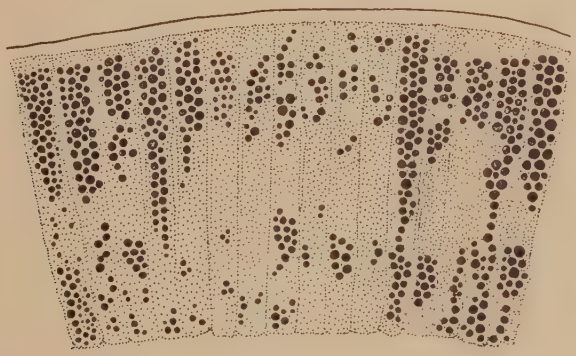

FIG. 7.—Vertical section of a portion of the duodenal epithelium of the plaice to which reference is made in Protocol (3). From the limb of a mucosal fold. Unstained preparation. Fixation by C.-K. Showing how the fat loading varies in different cells lying close together. The maximum load shown here is not the heaviest seen in this epithelium. × 1000.

but generally the granules extend close up to the clear cell border. In the troughs of the mucosal folds granules occur in a basal position within the cells also, where they are slightly larger. Large masses of granules also occur in the areolar tissue layer (c.p. with absence of osmicated fat).

Intestine.—The loading of the epithelium with osmicated fat is similar to but not as heavy as that in the duodenum. Smaller globules occur in the outer halves of the cells while the inner halves are clearer. The areolar tissue layer is devoid of osmicated fat.

Sudan-stained lipoid granules are just as abundant as in the duodenum, especially in the tips of the folds. But few granules occur in the areolar tissue layer.

Rectum.—Small fat droplets and globules occur in osmicated material but rarely. There is little absorption. Similarly in Ciaccio fixed material,

a few Sudan-stained granules occur in some cells, but the vast majority of cells are devoid of them.

Liver.—Much osmicated fat is present in the liver cells, the globules being of all sizes from very large to minute. But there are no lipoid granules present.

FIG. 8.—Transverse section of the intestine of the plaice referred to in Protocol (4). Unstained preparation. Fixation by C.-K. Showing the epithelium heavily charged with fat and also the fat-free areolar tissue layer. ×90.

(4) *Plaice, 23·3 cm. in length; given a mussel meal, interval of 48 hours, given an injection of olive oil emulsion (pH=8·3) through the mouth and killed 50¼ hours later. Fixation as before.*

Stomach.—Practically no absorption of fat or lipoids has taken place. Small osmicated globules and Sudan-stained granules occur but very rarely.

Duodenum and Pyloric cæca.—In osmicated material, the cells of the tips of the mucosal folds show a fairly heavy loading with fat globules. In the cells forming the limbs of the folds fat is not as abundant, the globules being confined chiefly to the central zones of the cells. Some folds are relatively free from fat. Fat is absent from the areolar tissue layer.

In Ciaccio-fixed material, there are no Sudan-stained, lipoid granules. These are absent from the epithelial and areolar tissue layers.

Intestine.—The epithelium is very heavily loaded with osmicated fat in all parts of the mucosa, i.e. in the tips, limbs and troughs of the folds (Fig. 8). The tips of the folds contain most fat, large globules completely

Fig. 9.—Vertical section of the epithelium forming the tip of a mucosal fold from the intestine of the plaice referred to in Protocol (4). Unstained preparation. Fixation by C.-K. Showing the maximal loading of the epithelium with fat. The size and distribution of the globules shown has been carefully treated. × 800.

filling the cells from the clear, outer hems to the basal parts. Near the bases of the cells the globules become smaller (Fig. 9). There is not a trace of osmicated fat in the areolar tissue layer (Fig. 8).

Ciaccio-fixed tissue shows the absence of Sudan-stained lipoid granules from all layers of the tract. In the epithelial cells, the vacuoles left after the removal of true fats by alcohols and xylol following the chroming treatment, are most conspicuous (Fig. 10). There is no trace of granules in the areolar tissue layer.

Rectum.—Neither osmicated fat globules nor Sudan-stained lipoid granules occur in any part of the epithelial or areolar tissue layers.

(5) *Plaice, 29·0 cm. in length; killed after fasting for 6 days. Fixation by Kolatschev's modified Champy method.*

Stomach.—Osmicated fat globules occur in all parts of the superficial

epithelium. In the parts lining the crypts of the gastric glands, the globules are few in number and localised in the basal portions of the cells (Fig. 11), while in the parts between adjacent crypts they occur also to some extent in the outer halves of the cells. The globules are generally much smaller than those occurring during fat absorption.

Occasionally a few minute globules occur in the mucus-producing cells forming the necks of the gland. But a small cluster of moderately large globules occurs in the basal cells of each tubule, these presenting a constant feature. The numbers vary, some cells possessing only one or

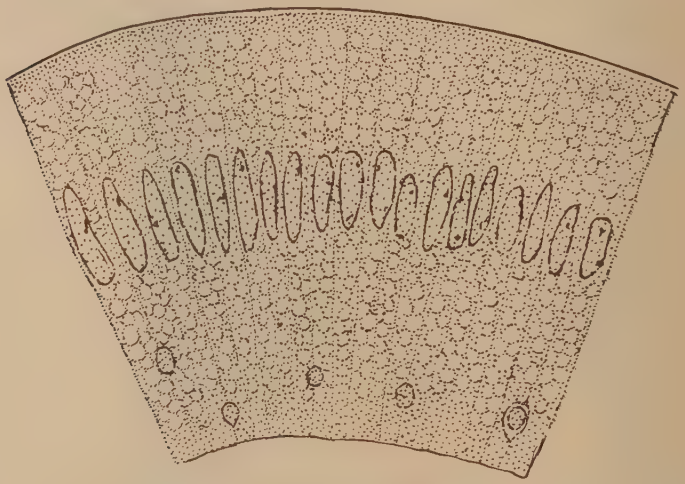

Fig. 10.—Vertical section of the intestinal epithelium of the plaice referred to in Protocol (4). Fixation by modified Ciaccio. Staining with Sudan and iron hæmatoxylin. Showing the absence of lipoid granules and the vacuoles left in the cytoplasm of the cells after removal of fats by the solvents employed in this technique. × 1300.

two, others six or seven, but every cell appears to contain fat (Fig. 11). There is no trace of fat in the areolar tissue layer.

All the fat described above is capable of extraction if sections are left overnight in turpentine.

The epithelial and areolar tissue layers of more posterior parts of the alimentary tract, i.e. of the duodenum and pyloric cæca, intestine and rectum, are free from fat and provide perfect blanks.

(6) *Plaice, 28·5 cm. in length; killed after fasting for 6 days. Fixation as before.*

Stomach.—Fat is present in the superficial epithelium, its distribution closely following that of the fat observed in the corresponding cells in the

29·0 cm. plaice described in Protocol (5). The neck-cells are similarly without fat, which is also absent from most of the deeper glandular cells. There is no fat in the areolar tissue layer.

Perfect blanks are obtained from all post-pyloric regions of the tract in every layer.

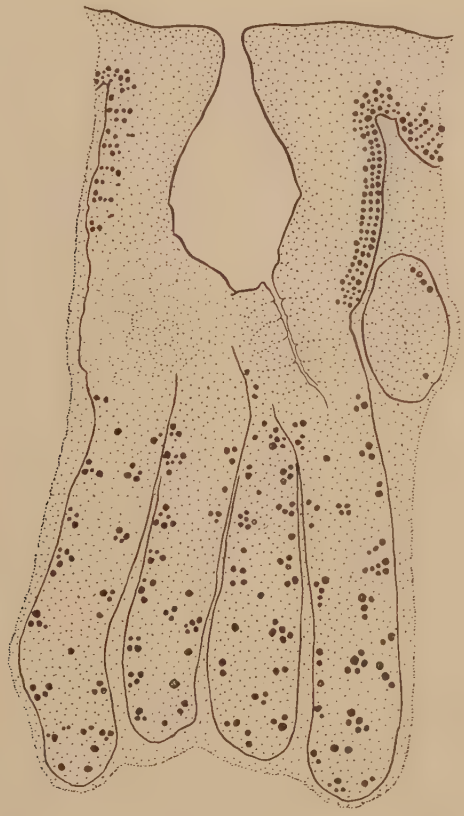

Fig. 11.—Vertical section of the gastric epithelium of the plaice referred to in Protocol (5). Unstained preparation. Fixation by C.-K. Showing the distribution of fat globules in the superficial epithelium, and the basal cells of the gland tubules and the absence of fat from the mucus-producing cells forming the necks of the tubules. ×500.

(7) *Plaice, 28·4 cm. in length ; killed after fasting for 7 days. Fixation as before.*

Stomach.—There is no osmicated fat in the cells of the superficial epithelium. The mucus-producing cells of the neck of the glands

similarly are without fat. But the basal cells of the gland tubules show fat globules as seen in the 28·5 cm. plaice of Protocol (6). The areolar tissue layer is without fat.

Note.—In a fish (32·5 cm.) killed after fasting for 6 days, Sudan-stained lipoid granules were absent from all regions of the alimentary tract.

Absorption of Fats and Lipoids in the Stomach.

The stomach of a plaice which has been fasted for six or seven days is usually collapsed and contains a small quantity of clear fluid apparently rich in mucin. Fat is to be found in the epithelial layer of all parts of the organ after such a fast. It occurs in the form of small globules localised chiefly in a basal position within the cells (in the epithelium lining the crypts) (Fig. 11), but also in the outer halves of certain cells (in the epithelium between adjacent crypts). The mucus-producing cells may contain each a few minute fat droplets but may be altogether without fat (Fig. 11). The basal cells of the tubules contain fat in variable quantities at the end of such a fasting period. In some cases every cell appears to contain a number of large globules, in others many cells may be without even droplets (Fig. 11). Fat does not occur in the areolar tissue layer.

In fish killed 30 hours after taking a single meal of Mytilus fragments, following on a period of fasting, the superficial epithelium shows an increased loading with fat globules. Only the outer $\frac{1}{5}$th part of each cell is consistently free from fat (Fig. 1). Below this zone fairly large globules are densely aggregated outside the nuclear zone, and rather smaller globules inside the nuclear zone extend to within $\frac{1}{5}$th of the depth of the cell from the base. The superficial epithelium lining the crypts of the glands shows a relatively lighter loading with fat, although more is present than after a period of fasting (c.p. Figs. 1 and 11). Fat is completely absent from the mucus-producing cells forming the necks of the tubules (Fig. 1), but globules occur in the basal cells of the tubules. After 50 hours of gastric digestion and absorption of Mytilus fragments, the loading of the superficial epithelium with fat is further increased (c.p. Figs. 3 and 1). The clear, outer zone is narrower and fat globules are so densely crowded into the outer halves of the cells as in many cases to render these parts solidly black in appearance under low powers of the microscope. Two or three longitudinal rows of globules are contained within a single cell, these rows extending closer to the basal margin of the epithelium than hitherto (c.p. Figs. 1 and 3). But there has been no passage of fat into the mucus-producing cells of the glands nor is there any sign of fat in the basal cells of the tubules. It is not possible to trace the fate of the fat observed in these latter cells after

a fast and during the earlier stages of digestion. It does not reappear while active digestion is taking place, so that it may be a metabolite during the process of digestion. The areolar tissue layer is devoid of fat droplets, so that if fatty elements are passed through this layer into the blood-stream, either there is no resynthesis of the elements or the resynthesised fat globules are so small as to be incapable of resolution under the highest powers of the microscope. After a single mussel meal then, the stomach is capable of fat absorption, the superficial epithelium being the most important element of the mucosa thus concerned.

In both cases of digestion and absorption of Mytilus fragments noted above, lipoid granules show a distribution similar to that of true fat globules. The granules are smaller in size, and although present in larger numbers they do not form the same bulk as the fat globules.

After the digestion of two mussel meals there is hardly a trace of fat in the superficial epithelium of the stomach, although considerable absorption has taken place in post-pyloric regions of the alimentary tract as shown by tissues subjected to precisely the same details of technique. In a fish 18·7 cm. long (not included in the protocols), which was supplied with six consecutive meals of Mytilus fragments liberally mixed with olive oil at 24-hour intervals and was killed 48 hours after the last meal, there was a loading of the superficial epithelium with fat globules yet not as heavy a loading as was obtained in the other fish killed about 50 hours after a single mussel meal. The probable significance of these findings is discussed briefly in the next part of this paper.

Absorption in the Duodenum, Pyloric cæca, Intestine and Rectum.

During a period of fasting, various changes occur in the condition of the alimentary tract of the plaice. Usually, as has been mentioned, the stomach is collapsed and empty or almost empty of fluid, but it may be filled to distention with clear fluid. The condition of the post-pyloric part of the alimentary tract also changes during such a period. The duodenum, which is the anterior half of that portion of the tract included between the pyloric and intestino-rectal valves, is usually moderately distended with clear, watery fluid but may be greatly distended or, on the other hand, contracted and empty. This also holds for the intestine and rectum. There appears to be some correlation between the conditions of the tract in the various regions, for when the duodenum is collapsed, the intestine is usually greatly distended, when the intestine is collapsed and empty, the rectum is full to distention. The changes from region to region at different times are similar to those occurring during the active

digestion of food, the same peristaltic activity of the walls of the tract being in evidence. The rectum assumes insignificant proportions when collapsed, but its walls are capable of greater distention than obtains in any part of the intestine anterior to the rectum. It holds a considerable volume of fluid at certain times, but whether the bulk of this fluid is removed by absorption or by way of the anus has not been determined, although it is shown that the rectal epithelium can absorb fats to some slight extent.

In fish supplied with a single meal following upon a period of fasting, changes similar to those mentioned above are observed. For a number of hours after the meal is taken, the stomach is greatly distended with food, while the more posterior regions are either distended with clear, colourless fluid or are collapsed and almost empty of fluid. In a number of experiments on the rate of digestion, the results of which are as yet unpublished, it was found that, at summer temperatures such as obtained when the observations on fat absorption were made, and when *Mytilus edulis* is the food supplied, the fluid in the duodenum is clear in fish killed 15 hours (16·7 cm.) and 16 hours (14·7 cm.) after taking a single meal. But in fish killed $18\frac{1}{4}$ hours (20·1 cm.) and $18\frac{1}{3}$ hours (15·8 cm.) after taking a single meal, the fluid in this part of the tract is yellowish in colour and contains shreds of finely divided solid food, which are seen to be in the act of passing through the pylorus. Thus the first slight relaxation of the sphincter takes place at from 16 to $18\frac{1}{4}$ hours after taking a single meal. Such times as have been noted are likely to vary with variations in temperature, but with this we have no concern at present. It is interesting to note, however, that when Nereis is supplied as food but other conditions identical, the times were different. After $22\frac{1}{2}$ hours (19·0 cm.) the stomach is greatly distended and the duodenal fluid clear, but after 24 hours (21·5 cm.) the duodenum contains brownish fluid but no solid, the stomach being greatly distended. With Nereid worm as food, therefore, the sphincter is not relaxed until almost 24 hours have elapsed since the taking of food. The results given above are taken from a much larger mass of data.

Then it was found that the stomach of the plaice retains food for a period of from 40 to 48 hours after the time of taking food. At the first relaxation of the sphincter and for a number of hours afterwards, only fluid and finely-divided solid particles, forming some at least of the products of the gastric digestion of Mytilus or Nereis fragments, are allowed to pass through into the duodenum, the relaxation being incomplete. Total relaxation of the sphincter occurs at summer temperatures at 40–48 hours after the taking of food, when large and relatively undigested fragments of food are passed into the duodenum.

The time taken for food to pass along the duodenum into the intestine

is probably short, but no doubt considerable variations occur ; $18\frac{1}{2}$ hours after taking a mussel meal a plaice shows duodenal contents of a straw colour containing shreds of finely divided solid matter, while clear, colourless fluid fills the intestine (20·1 cm.) ; $19\frac{1}{4}$ hours after a meal is taken, the intestinal contents are coloured like those of the duodenum (19·4 cm.). In fishes killed 21, $21\frac{1}{2}$, and 27 hours after taking a mussel meal, the intestine was full of brown fluid containing shreds of solid, while in fishes killed $27\frac{3}{4}$ and 30 hours respectively after taking food, the anterior part of the intestine was considerably distended with brown fluid, the posterior part being contracted and empty. Only fish originally filling the stomach with food were considered in any of the cases mentioned above. In view of these results, it appears that absorption in the intestine commences within an hour or two, or at most a few hours, of duodenal absorption, and long before the stomach is completely emptied of the solid food remaining from the single meal.

Food appears to stay in the duodenum and intestine for a variable period. In all fish killed 30 or fewer hours after taking a meal only clear fluid was contained in the rectum. In a fish (14·4 cm.) killed $38\frac{1}{2}$ hours after taking a meal intestine and rectum alike contained brown fluid and finely divided solid. In almost every fish killed after a longer period of digestion the rectum contained fluid coloured with mussel food. In a 16·9 cm. fish killed $39\frac{3}{4}$ hours after taking a meal, the intestine was contracted and empty, its walls appearing yellow and opaque, while the rectum was distended with brown fluid and finely divided solid. It is seen then that partly digested food remains in the duodenum and intestine for more than 20 hours and that a part of the food of the single meal is passed into the rectum as fluid or finely divided solid before complete relaxation of the pyloric sphincter occurs, i.e. whilst solid food remains in the stomach. The time taken for complete removal of a single meal is not easy to determine. Only finely divided mussel fragments were observed in the posterior part of the tract towards the end of the meal. After 54 hours food particles and brown fluid still remain, but after 60 hours only clear fluid remains in every part of the tract.

Summarising these results for a single mussel meal and taking times from the moment the stomach is filled with food, the following approximations are obtained :—

1. Partial relaxation of pyloric sphincter after from 16–18 hours.
2. Finely divided solid and fluid food reaches intestine after 19 hours.
3. Food particles first reach rectum after 38 hours.
4. Complete relaxation of pyloric sphincter after from 40–48 hours.
5. Alimentary tract finally cleared of food after from 54–60 hours.

TABLE I.

Passage of Food through Alimentary Tract of Plaice: 2 Meals of Mytilus Fragments.

Length of fish (cm.).	Time after 1st meal (hours).	Time after 2nd meal (hours).	Food in Stomach (gm.). From 1st meal.	From 2nd meal (carmined).	Food in Duodenum and Intestine. Nature of food.	Weight of solid (gm.).	Nature of rectal contents.
27·0	28	$1\frac{1}{4}$	3	7	Br.F. & F.D.S.	–	Br.F. & F.D.S.
23·1	$29\frac{3}{4}$	3	3	4·5	Br.F., F.D.S. & S.	2	Br.F.
23·4	$31\frac{1}{2}$	$5\frac{1}{4}$	7	2·5	Br.F. & F.D.S.	–	C.F.
24·4	$45\frac{3}{4}$	19	Nil	5	Br.F., F.D.S. & S.	4	C.F. (1·5 gm. S. passing in)
22·3	$49\frac{1}{4}$	$22\frac{1}{2}$	Nil	3	Br.F., F.D.S. & S.	3	C.F. & F.D.S. passing in.

Br.F.= Brown fluid. F.D.S.= Finely divided solid. S.= Solid. C.F.= Clear fluid.

A few experiments were carried out to show what changes, if any, occur in the rate of passage of food through the alimentary tract when 2 meals are supplied on consecutive days, following a period of fasting. The series is incomplete, but the results are sufficiently conclusive to merit their being presented in the accompanying table. The food was supplied in the form of pieces of mussel weighing about 0·2 gm., the pieces forming the second meal being covered with carmine particles. Although a cloud of carmine particles pass out through the gills when the fish swallows the food, yet in every case it was possible to distinguish the second meal from the first in the gastric contents. In all these instances presented, the pylorus had been partially relaxed presumably before the second meal was presented, so that the finely divided solid and brown fluid found in the duodenum and intestine in each case is to be expected. Also, since with a single meal the pyloric sphincter is completely relaxed after 40–48 hours, it is partly anticipated that none of the particles forming the first meal in the last two instances will remain in the stomach. But differences are seen in the rate of progress of the first meal along the tract. It is seen that after $29\frac{3}{4}$ hours much of the first meal has passed into the duodenum as large particles of solid. And in two instances food has passed into the rectum after 28 hours, while in another, solid food is passing into the rectum after $45\frac{3}{4}$ hours. These results although incomplete suggest that food from a meal passes along the tract more rapidly if a second meal is taken. If completely established the point would be of great significance, since it would be implied that the more frequently the fish takes food, the less efficient it becomes, or more precisely, a fish feeding once per day is less efficient than one taking food on alternate days or less frequently, assuming that digestion of large food particles is also a measure of absorption.

It is evident from the above results that if a fish is killed 30 hours after taking a meal of Mytilus fragments, following upon a period of fasting, partly digested food materials have been present in the duodenum, in fluid and finely divided solid condition, for approximately 12 hours, and in the intestine for an hour or two, or at most several hours less than 12. These conditions hold then for the fish to which reference is made in Protocol (1). In this fish it was seen that only the epithelial cells forming the tips of the mucosal folds have undertaken the absorption of fats and lipoids. These cells show lipoid granules and minute brown-stained osmicated particles crowded into their outer halves. There are no granules or fat particles in the cells of the limbs and troughs of the mucosal folds, so that absorption apparently commences in the parts of the mucosa which are likely to project furthest into the lumen of the distended gut.

In a fish killed 50 hours after taking a mussel meal, the duodenum and intestine must have contained food materials, which may or may not have been partly digested in the stomach, in the form of fluid and finely divided solid particles for about 30 hours. In addition, food remaining in bulk after complete gastric digestion has taken place was passed into the duodenum a number of hours (less than 10) previously. These conditions hold then for the fish to which reference is made in Protocol (2). In the duodenum of this fish, fairly dense aggregations of minute fat droplets occur in the cells of the tips of the mucosal folds and groups of cells contain clusters of fairly large globules. In the intestine, what is probably the earliest histologically demonstrable stage in true fat absorption is seen. Extremely minute osmicated droplets occur in a constant position within the cells at about $\frac{1}{3}$rd of the depth from the clear, outer boundary (Fig. 4). But in corresponding material the whole of the outer halves of the cells from the clear margin to the nuclear zone is crowded with minute lipoid granules (Fig. 6), which are thus more numerous than true fat droplets and also have a wider distribution.

In the case of the fish referred to in Protocol (3), the stomach was distended with carmined Mytilus fragments of the second meal (about 5 gm.). The duodenum was distended with brown fluid and fine solid, but also contained about 4 gm. of coarser solid. The posterior part was contracted but the intestine was distended, as if food was in the act of passing backwards, which view was supported by the fact that solid particles were passing through the intestino-rectal valve into the clear rectal fluid. The walls of the anterior half of the intestine and the whole duodenum appeared yellow and opaque. Food could not have been in contact with the epithelium of the duodenum and intestine for as long a period as with that of corresponding regions of the fish referred to in Protocol (2), since the times from the taking of the initial meal were

45¾ hours and 50 hours respectively. But more material had been available for duodenal and intestinal absorption, since a portion of the second meal had been passed over. Accordingly an increased degree of absorption might reasonably be anticipated, but not more than twice the amount seen after a single meal. It is to be noted that the mussel samples used in these meals were kept as constant as possible, and that several pieces of tissue from different parts of the same organ were fixed at one and the same time. Comparisons of the data presented briefly in Protocols (2) and (3) show conclusively that the increase in absorption in the post-pyloric part of the tract is very great when two meals are supplied, much more than twice as great.

After two meals, the cells of the tips and limbs of the mucosal folds of the duodenum and intestine, and especially the former, are very heavily loaded with fat globules, so that the outer halves of the cells forming these parts of the folds are rendered solidly black. Only the clear cell margins are devoid of globules. So heavy is the loading that it is not possible to show the detail in a text-figure. The tissue shown in Figure 7 was from a part of the epithelium showing relatively little fat, and was selected to show how cells vary in their fat content. In the case of lipoid granules, there is no considerable increase in numbers within the epithelial layer, but masses of granules occur in the areolar tissue layer, showing that increased absorption of lipoids has ensued.

When it is remembered that after two mussel meals there was little fat and lipoid material in the gastric epithelium, an explanation is afforded for the increased absorption of these materials in the duodenum and intestine. The marked decrease in gastric absorption and equally well-marked increase in intestinal absorption strongly suggest that the absorptive function is largely transferred from the stomach to the duodenum and intestine when two meals are taken in quick succession, and that the stomach plays a greater part as an organ of absorption when rations are restricted, the post-pyloric intestine assuming the rôle of principal agent of absorption when food is abundant.

The quantity of fat in Mytilus flesh is not very great. According to the most recent analyses (Daniel and Doran (1926)), the fat in the wet flesh of *Mytilus edulis* ranges from 2·0 per cent before spawning to 0·5 per cent immediately after spawning. The wet flesh also contains cholesterol and probably highly unsaturated fatty acids.

According to Bolles-Lee, not only fats but also cholesterin-fatty acid mixtures are affected by osmic acid following upon treatment with bichromate. Cholesterin alone is unaffected. It appears, therefore, that the droplets browned and blackened by osmic acid during Champy fixation of tissues taken from fish which have digested mussel fragments, are either true fat or cholesterin-fatty acid mixtures. In materials fixed

by the Ciaccio method used, some of the Sudan-stained granules are undoubtedly those affecting osmic acid also. It may be that these common elements are cholesterin-fatty acid mixtures. It is assumed, out of a consideration of the chemical composition of the food, that Sudan-stained granules not evident in osmicated preparations are granules of cholesterol.

As has already been mentioned, various true fat meals were supplied to certain fish. The fish to which reference is made in Protocol (4) was given an injection of olive oil emulsion prepared by adding phenol phthalein to olive oil and titrating with standard soda to a faint pink (pH=8·3). The alimentary tract had not contained food for two days previously and two days elapsed further before the fish was killed. Thus the absorption of pure oleic fat is being considered. The intestinal epithelium is very heavily loaded with fat in all parts, i.e. in the tips, limbs and troughs of the mucosal folds (Fig. 8). The loading is more consistently heavy than is that in any part of the post-pyloric intestine after two mussel meals. The cells are crowded with osmicated globules of all sizes, those of the mucosal tips being especially densely crowded (Fig. 9). It appears from the evidences afforded, that although there is no histological differentiation in any parts of the post-pyloric epithelia, yet the tips of the mucosal folds play a greater part in fat absorption than do other parts, since absorption commences here and is also more intense.

Lipoid granules are completely absent from all parts of the tract in any region (Fig. 10). It was inferred above that the technique employed favours cholesterin and cholesterin-fatty acid mixtures in Mytilus-fed fish. But it probably affects other lipoids also. The absence of lipoid granules has a special significance. Recently (1929), Sinclair investigated the rôle of phospholipids in fat absorption in mammals, from the chemical point of view. Upon evidence he collected he put forward the hypothesis that, "within the epithelial cells of the intestinal mucosa there is a 'specific' phospholipid which occupies an intermediary position between fatty acids and neutral fat, as represented by the equation :

$$\text{Fatty acids} \rightleftharpoons \text{Phospholipid} \rightleftharpoons \text{Neutral fat.}$$

It is an invariable characteristic of this process that the *amount* of phospholipid remains constant. As soon as fatty acids are absorbed into the epithelial cells molecules of the 'specific' phospholipid react with the free fatty acids (or soaps) to form neutral fat ; immediately, however, the phosphoric acid-base complex unites with absorbed fatty acids and glycerol to form phospholipid, thereby maintaining the amount constant." The synthesised molecules of neutral fat coalesce to form droplets.

It follows from the above that phospholipid material should be present in cells fixed during active absorption of fats at any time, if only in a form not capable of resolution under the microscope, and especially in cells which have not taken up and resynthesised the cleavage products of fats. In the latter cells it is not unreasonable to anticipate the presence of phospholipid bodies of resolvable size. But in the cells of the intestinal and duodenal epithelium (and also the gastric epithelium) granules such as are preserved with the lipoid technique used are completely absent. It may be that the technique does not favour the "specific" phospholipid, or that the groups of molecules are too small to be visible under the highest powers of the microscope; but in any case, it is not possible to present evidence which supports this hypothesis.

In the duodenum of the emulsion-fed fish, the epithelial cells show relatively less fat than those of the intestine. Fat droplets and globules present in the tips and limbs of the folds are localised in the nuclear zone of the cells, the outer and basal halves of the cells being clear. This is taken as indicating the final stage in fat absorption, when the cells are becoming relieved of the fat they have taken up and when the cleavage products of fats are no longer being presented to the cells from the materials remaining in the lumen of the alimentary tract. Briefly recapitulating the stages observed in fat absorption in the post-pyloric part of the alimentary tract, we have :—

1. The formation of minute fat droplets in a constant cytoplasmic zone about $\frac{1}{3}$rd of the depth of the cell from the clear cell border (Fig. 4).
2. The formation of minute droplets between the above-mentioned zone and the clear cell border, with contemporary enlargement of the droplets noted in (1) (Fig. 5).
3. The coalescence of minute droplets into globules with which the cells become crowded in all parts, larger globules occurring in the outer halves of the cells (Figs. 7 and 9).
4. The removal of fat in all parts of the cells except the region of the nucleus and the final clearing of the cells.

Only in one or two instances was fat noted in the areolar tissue layer and in these only very few small clusters of extremely minute droplets. From the results thus obtained it is concluded that the cleavage products which pass out of the epithelial layer into the areolar tissue layer are not resynthesised into fats in this layer, unless the process of resynthesis ends with the production of fat droplets so small as to be incapable of resolution under the highest powers of the microscope. Even most minute

droplets appear to be absent. It is beyond the scope of this work to suggest that the cleavage products are passed on to the liver as such, but this appears possible. Lipoid granules are commonly seen in the areolar tissue layer after a mussel meal or meals. And in several fishes not mentioned in the Protocols of this paper the liver contains much fat after a meal, but lipoid granules are completely absent. The blood from the digestive organs is conveyed to the liver by the hepatic portal veins directly, so that either there is no storage of these lipoids in the liver or some chemical transformation takes place in this organ.

The rectum is capable of some slight degree of fat and lipoid absorption, but is not a typical organ of absorption. Small clusters of fat globules and lipoid granules are to be found in some of the rectal epithelial cells during digestion and absorption of a meal. But the quantity of these substances present is negligible from the point of view of practical ends attained.

There is no storage of fat in any part of the post-pyloric division of the alimentary tract.

SUMMARY AND CONCLUSIONS.

1. Fat occurs in all three types of cell composing the gastric epithelium at the end of a period of fasting extending over 6 or 7 days, but is completely absent from the mucosa of all post-pyloric regions of the alimentary tract at such a time.

2. There is a marked increase in the fat content of the superficial epithelium of the stomach after 30 hours and 50 hours of gastric digestion, the stomach being thus shown to be an effective organ of fat absorption.

3. Considerable quantities of fat are present in the duodenal and intestinal epithelia after fat-containing meals have been digested. The rectum is capable of some slight absorption, although its capacity in this respect is very much smaller than that of the duodenum or intestine.

4. It is suggested that there is a transference of the function of fat absorption from the stomach to the post-pyloric intestine when the frequency with which meals are taken is increased.

5. Globules of true fat are not typically observed in the areolar tissue layer of the alimentary tract, although lipoid granules may occur in abundance. It is suggested that resynthesis of the cleavage products of fats does not occur in this layer.

6. Data are presented bearing upon the rate of digestion of *Mytilus edulis* and Nereid worm.

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Growth and Maintenance in the Plaice (*Pleuronectes platessa L.*). Part I.

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With 19 Figures in the Text.

CONTENTS.

		PAGE
	Introduction	103
	Methods	106
I.	Results of Preliminary Experiments at Cawsand, 1928	108
	1. Minimum Requirements and Maintenance	108
	2. Maximum Requirements and Growth	110
	3. Growth and Intermediate food supplies	112
	4. Conclusions drawn from the Experiments of 1928	113
II.	Objections to the Form of the Preliminary Experiments	114
III.	Modification of Apparatus and Methods (1929)	117
IV.	Results of the Cawsand Experiments of 1929	118
	1. Minimum Requirements and Maintenance	118
	2. Maximum Requirements and Growth	121
	3. Conclusions drawn from the Experiments of 1929 (Cawsand)	130
V.	Results of the Lympstone Experiments of 1929	131
	1. Introduction	131
	2. Minimum Requirements and Maintenance	132
	3. Maximum Requirements and Growth	135
	4. Conclusions drawn from the Experiments of 1929 (Lympstone)	142
VI.	General Discussion of Results	142
VII.	Tables 1–54	148–174

Introduction.

The experiments to be described below were proposed by the Ministry of Agriculture and Fisheries and were carried out under the auspices of the Marine Biological Association, being subject to the direction of Dr. E. J. Allen, F.R.S., and Dr. E. S. Russell. Mr. Harvey, hydrographer to the Association, directed the construction of the sea-water pond and the fish boxes during the early stages of the preliminary work and assisted in organising supplies whenever alterations were made to the apparatus.

The aim of the experiments is to determine the food requirements of the plaice with respect to the maintenance of life apart from growth, and

to ascertain what quantity of food is available for purposes of growth after basal requirements are satisfied, in the case of an actively feeding fish during its third season of growth. The work thus affords an

FIG. 1.—View looking out over the sea-water pond at Pier Cellars into Cawsand Bay, showing the constructed sea-wall with its grating. The hawser system is shown and the boxes are seen in situ in the neck of the pond. Only a portion of the pond is shown, but the low-water limits are, roughly, those seen in the picture.

indication of the growth-rate of a 2-year old plaice and also the relation existing between the quantity of food taken and growth.

The investigations were carried out at Pier Cellars, which lies on the west shore of Cawsand Bay and thus immediately outside Plymouth

Sound. Here, a rectangular cove some 120 feet by 70 feet opened on its northerly aspect, by a narrow neck roughly 20 feet wide, into Cawsand Bay. During the spring of 1928, when Mr. H. O. Bull was in charge of the work, this narrow neck was closed by a reinforced concrete sea-wall provided with a strong grating of vertical bars, horizontal girders, and stout $\frac{1}{2}''$ mesh galvanised iron wire-netting. The grating measures 12 feet by 9 feet, and the total depth of the sea-wall is 15 feet, so that, as the grating is fitted into the upper part of the wall, with its long axis horizontal, a depth of 6 feet of water is accommodated in the cove at low water, to provide a permanent sea-water pond (Fig. 1). The pond possesses a shingle beach at its south end so that between mid-tide and low water

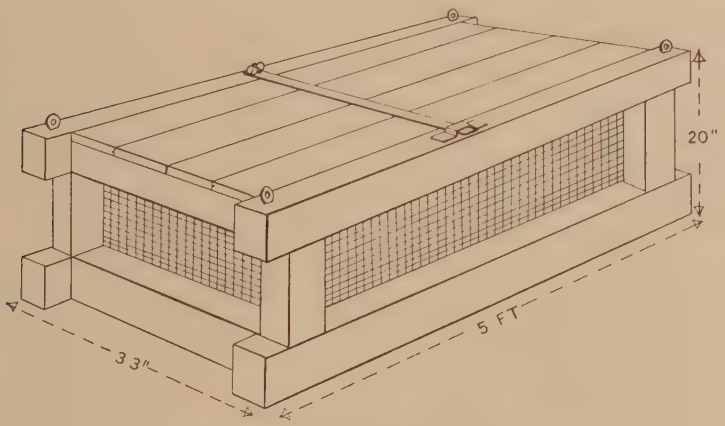

FIG. 2.—Diagram of a fish box as used in the Cawsand preliminary experiments of 1928.

the depth of sea-water in it shallows from about 6 feet in the neck to nil on the beach. The depth of the portion of the wall below the grating was so arranged that the sea enters the pond about 3 hours before high tide and finally leaves it about 3 hours after high tide. Thus each day is divided into 4 parts of approximately six hours duration each. During the first period, say, the pond is in open communication with the sea outside, for the tide is either running into it or ebbing out of it. During the second period, the water in the pond is quiescent at its lower level, the tide being off the wall outside. The third and fourth periods each day are repetitions of the first and second periods of course. By this means, the pond is provided with an adequate circulation of water, for, with a spring tide rising to 16 feet above the Plymouth datum line, roughly 7 feet 6 inches of water flows over the wall through the grating, bringing the depth of water in the deepest part of the pond to about 13 feet 6 inches.

Two steel wire hawsers were stretched out from end to end of the pond and over its surface, passing along the narrow neck. These were fixed at one end to the bars of the grating, at the other end to stout pins fixed vertically in the beach. The boxes into which the fish were placed subsequently, were slung on this hawser system, so that they floated at the surface of the water, as shown in Fig. 1.

Each box, with inside dimensions of 4 ft. 6 in.$\times$2 ft. 3 in.$\times$1 ft. 6 in., consisted of a strong deal framework and was provided with a solid bottom and a solid but halved, hinged lid, secured by means of hand bolts and a wrought-iron cross-bar fitted with a staple and a bolt and wing nut. The vertical faces of the box consisted during the early stages of the experiments of $\frac{1}{2}$-inch mesh galvanised iron wire-netting to ensure an adequate circulation of water through the box. Fig. 2 shows one of the boxes. Towards the end of the year 1928, when there appeared some likelihood of the boxes being damaged during gales, the framework of each box was further strengthened with wrought-iron plates and cross bolts, and an additional hawser system was introduced. But auxillary floats were employed in order to keep the boxes at the surface of the sea.

METHODS.

Upon completion of the preliminary arrangements, a number of 2-year old plaice were trawled in Cawsand Bay or in the nearby vicinity, kept under circulation on board s.s. *Salpa* and placed into storage boxes in the pond at Pier Cellars as promptly and carefully as possible. These fish were fed daily and were observed frequently for a number of days following their capture by Mr. H. O. Bull, who removed any individuals showing signs of ill-effect due to trawling or any other cause. From the remaining fish, 36 individuals were selected, this number being equally divided between the sexes. From the 18 fish of each sex three groups each containing 6 individuals were formed, each group being housed in a separate box. Thus 3 boxes each contained a group of 6 male fishes, and 3 boxes each a group of 6 females.

Subsequently, at the commencement of the preliminary experiments, one group of fish of each sex was provided with minimum rations, an attempt being made to satisfy basal requirements without allowing growth, i.e. to keep the length and weight of the fish constant. Trial and error methods together with periodic adjustments gave a reasonable measure of success. Further, one group of fish of each sex was supplied with maximum rations, arranged to satisfy the appetite of the fish and to allow full growth. The third groups of fish of each sex were given rations intermediate between the minimum and maximum ones.

The food supplied to the fish consisted of the best specimens of *Mytilus*

edulis obtainable, opened, allowed to drain for 30 minutes, and then cut up into pieces of suitable size. Small particles of food were studiously avoided so as to prevent undue loss. In the description which follows,

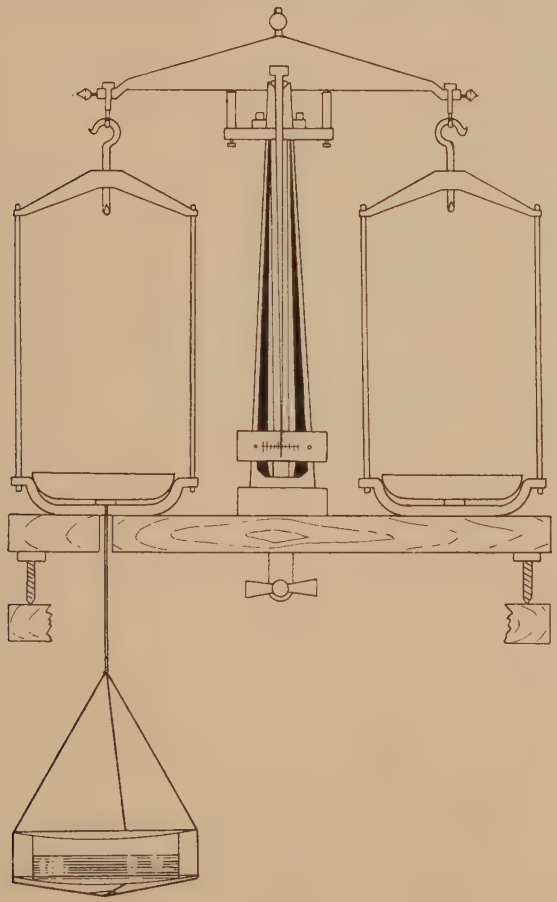

Fig. 3.—Diagram showing the weighing apparatus. The two large clock glasses and the shallow glass dish containing sea-water are slung from the left side of the balance by means of a wire basket.

mention of food implies food of the nature mentioned above. Food rations were presented to each group of fish daily as consistently as possible and at a time when the water in the pond was in a quiescent state, so that the chances of food being washed away by a rising tide were minimal ones. The times of feeding were noted and daily records were

kept of the temperature of the water in the vicinity of the boxes. A look-out was maintained for parts of rations uneaten and the behaviour of fish upon presentation of food was noted.

Measurings and weighings of the fish were carried out fortnightly, and always, the time that had elapsed between the last feeding operation of the fortnight and the time of weighing was maintained constant. The actual weighing was carried out in water, the apparatus used being shown in Fig. 3. An approximately constant weight of sea-water was placed in the glass vessel attached to the left arm of the balance for each weighing, the vessel then being closed and the weight of the whole apparatus taken. After measuring the length of a fish, it was carefully blotted with a clean, dry glass-cloth and inserted with equal care into the closed vessel. After a few preliminary attempts at thus inserting a fish, the operation can be performed without the loss of even a trace of water, when care is taken. A second weighing gives the weight of the apparatus plus a single fish, from which, by subtraction of the first weighing, the weight of the fish is obtained. By repeating with the other members of a group, the weights of all the individuals are obtained. Fish were measured to the nearest 0·1 cm., and were weighed to the nearest 0·5 gm. The balance used is by Oertling (Fig. 3).

I. RESULTS OF PRELIMINARY EXPERIMENTS AT CAWSAND, 1928.

1. Minimum Requirements and Maintenance.

The results of the experiment with a group of male plaice fed with minimum rations are shown in Table 1. The attempt made to maintain the fish at a constant weight met with some success, as is seen. The mean fish increased its initial weight of 42·0 gm. by only 4·8 gm. during the 176 days of the experiment, of which increase, 4·2 gm. was obtained during the last 39 days. The average quantity of food taken daily by the mean fish varies, but for the most part between such narrow limits as 0·6 gm. to 0·7 gm. But closer inspection of Table 1 shows that the results of the experiment are modified by a remarkable event. It is seen that the fish designated A1 steadily declined during the course of the experiment, and gradually lost weight until late November, when it died.

It was observed during feeding operations that these male fish generally displayed avidity for food, rising towards the surface of the water as soon as it was presented, and even snapping at each other at feeding time before food actually was offered to them. As soon as the lid of the box was opened, the fish were seen to be in great readiness to take food. But the fish A1, and during the early stages of the experiment A5 also,

was invariably too slow to obtain food. After a short time it made no attempt to compete with its fellows, but steadily sulked in a corner of the box, watching the feeding attempts of the others. It was completely subjugated and, as the rations were taken by the other fish immediately they were placed in the water, it is doubtful if it succeeded at all in obtaining an appreciable quantity of food. Because of this factor, it seems reasonable to suggest that the fish A1 be excluded from consideration in the preparation of weight-length averages and mean maintenance ration estimates. It must be observed here that although individual values are arranged in order in Table 1, yet it was not possible to recognise each individual with certainty during the course of the experiment, and the order presented is not the only possible one. But certain fish are definitely recognisable, e.g. A1, which was originally the longest fish in the group and which came to be distinguished by its slight weight, and A5, the smallest fish of the group.

The values shown on the right-hand side of Table 1 are obtained when the fish A1 is excluded from consideration. There appears to be reason for maintaining that they are more accurate than values which take this fish into consideration. During the course of the experiment, the mean fish increased its initial weight of 41·6 gm. by 5·2 gm., while the daily food allowance varied, for a greater part of the time between 0·7 gm. and 0·8 gm. Thus, if the suggestion made above is accepted, the value for the daily maintenance ration obtained from data concerning the whole group must be increased by 0·1 gm. Between August 14th and November 30th the corrected daily ration varies between 0·017 and 0·019 of the average body-weight for each period, and may be taken as approximately constant at 0·018 for a 42·0 gm. mean fish. During December and the early part of January the fraction of the average body-weight which must be supplied in food per day, which fraction will subsequently be referred to as the maintenance ratio, falls appreciably. As is shown in Table 1, during the last 39 days of the experiment, the corrected mean fish increased its weight by 4·2 gm. as against 1·0 gm. for the previous 137 days on a reduced ration per day corresponding to 0·014–0·015 of the average body-weight.

The results of the experiment with a group of female fish fed with minimum rations are presented in Table 2. The problem of the decline of certain individuals, due to lack of success in the competition for food within the minimum groups, is obvious, if not as well marked as in the cases of the males. But the declining females B1 and B5 undoubtedly were able to obtain food, since for periods up to 32 days their body-weights were maintained constant. These fish showed the same disregard for food as did A1 amongst the males, but it is impossible to correct the mean values obtained for the whole group, since for periods they undoubtedly

must have obtained food. In great probability, the mean values shown are higher as regards food quantities than the true values desired.

The females were supplied with rations similar to those given to the males, and because of the difference in size were thus allowed more than sufficient for purposes of maintenance. Thus, during 147 days from August 14th, a daily allowance of rather more than 0·6 gm. on the average resulted in an increase in body-weight of the mean fish of 15·7 gm. It is seen from Table 2 that during the period ending July 30th, a daily allowance of 0·5 gm. of food resulted in an increase in body-weight of 0·9 gm., while in the following period 0·4 gm. of food per day enabled the body-weight to be kept approximately constant. It appears likely that for female plaice of 25·0 gm. body-weight, a daily food allowance of 0·4–0·5 gm. would prove adequate. The maintenance ratio would lie between 0·016 and 0·020, and would compare fairly closely with that obtained for males.

2. Maximum Requirements and Growth.

Data concerning fully growing male fish are presented in Table 3. After 176 days of growth the mean fish had increased its initial size of 16·0 cm. and 38·8 gm. by 6·6 cm. and 86·0 gm. The initial length is thus increased by rather less than half its value while the initial weight is rather more than tripled. The total quantity of food taken is about 1129 gm., or approximately 14 times the average body-weight for the whole period, and an average of about 6·4 gm. per day. Of the whole quantity of food consumed, an uncertain quantity had been used for purposes of maintenance. It is not possible to know the exact amount of food necessary to maintain this mean fish, since the values determined experimentally for the single set of males kept on minimum rations do not correspond to those of fully growing fish, as the maintenance requirements must vary with size. But an approximation will be obtained if it is assumed, in the absence of experimental data in these preliminary experiments, that the ratio, spoken of as the maintenance ratio (M), is constant for fish of any size within the range of these experiments. In Table 3 are given estimations of the maintenance requirements of the mean, fully growing male fish, the maintenance ratio being taken from the corresponding periods shown in Table 1. These estimates are probably high, expecially during the latter stages of the experiment, because it is likely that the value of the ratio will fall with increase in size of a fish. But this rough estimate indicates that approximately $\frac{1}{5}$th part of the total food consumed is used up in satisfying the maintenance requirements of the mean fish, only $\frac{4}{5}$ths of the total being available for purposes of growth. About 900 gm. of food used for growth purposes produced 86 gm. of mean fish, so that for

the whole period the fish requires 10 to 11 gm. of food over and above the maintenance ration to increase its weight by 1·0 gm.

It is seen from Table 3 that the increments of growth in weight are much more irregular than the corresponding food increments. Thus, for the three periods included between August 14th and September 26th, the average quantity of food consumed per day falls slightly in successive periods, while the increase in weight falls for the second period to rise again in the third. There is also a striking acceleration of growth in weight during early December which appears disproportionate to the quantity of food taken. This may be due in part to a fall in the quantity of food necessary for pure maintenance allowing of more food being available for purposes of growth. But it is probably also dependent upon other factors, as a glance at the results for the period ending January 8th will serve to show.

The results of the experiment with fully growing female fish are more complete than those concerning corresponding males. There was no accident such as interfered with the results in the latter case, when a box was forced open and three fishes gained their freedom. From Table 4, it is seen that the mean female fish was initially 16·1 cm. in length and 43·3 gm. in weight, and was finally 23·3 cm. long and weighed 151·2 gm., showing an increase in length of 7·2 cm., in weight of 107·9 gm. Considerable variation in growth occurs, for while the initial weight of D4 is less than tripled, that of D5 is quadrupled. It is interesting to note that during a whole growth-season a small initial difference in weight between two fishes may become considerably amplified. Thus in an environment holding an abundance of food, an initial difference of 13 gm. in the body-weights of the fishes D5 and D6 (Table 4) results in a difference of 90 gm. in these weights at the end of the growth-season. The same fact is shown by comparison of the initial and final body-weights of D2 with those of D5.

During the entire period, the mean fish takes 1129 gm. of food—rather less than 12 times the average body-weight for the period, and an average of 6·4 gm. per day. It appears likely that the females make better use of food for purposes of growth than do the males, since 1129 gm. of food produce 107·9 gm. of mean female fish as against 86·0 gm. of mean male fish, when the initial sizes were comparable.

No attempt has been made to estimate the maintenance requirements of the fully growing female fish. It was noted above that considerable growth took place in the mean female fish supplied with maintenance rations. Moreover, the selection of fish at the commencement of the preliminary experiments was such that, despite considerable growth, the females given minimum rations were smaller at the end of the experiment than were the females given maximum rations at the commencement.

But, assuming that the maintenance ratio is approximately constant at from 0·018 to 0·020, the maintenance requirements of the fully growing female fish will range from 200 gm. to 245 gm. of food for the whole period. This rough estimate is suggested tentatively.

3. Growth and Intermediate Food Supplies.

As was mentioned above, groups of male and female fish, each containing six individuals, were supplied with quantities of food intermediate in value between the minimum and maximum rations. This intermediate ration was about $\frac{1}{3}$rd of the maximum one. The results of the experiment with males are shown in Table 5, where it is seen that the mean fish increased its initial size of 16·0 cm. and 42·5 gm. by 3·8 cm. and 51·1 gm. during the 176 days of the experiment. During this period it took 393 gm. of food, an average of 2·2 gm. per day. Table 7 shows initial lengths and weights, final lengths and weights and the total quantities of food consumed by each of the three groups of each sex. It is seen that in maximum-fed males, 13·3 gm. of food are required to produce 1·0 gm. of fish on an average for the whole period of 176 days. In the case of intermediate-fed males, only 7·7 gm. of food is required to produce the same quantity of mean fish. Thus there appears to be some difference in efficiency of the two groups. This question will be returned to later.

Returning to Table 5, it will be seen that the initially larger fish fared much better than did the smaller ones, c.p. E1, E3, and E4 with E2 and E6. Ultimately, the group comes to consist of a mixture of " maximum " and " minimum " fish. A problem comparable with that of the decline of individuals in the minimum groups thus arises, due to the competition for food existing between the members of the group. It was observed during feeding operations that certain individuals took liberal helpings of food while others made no attempts to obtain it while under observation. It was made a rule after a short time to close up the box immediately following the presentation of food in order to eliminate the possibility of fish refusing to take food in the presence of the observer. But in spite of such a precaution, certain individuals soon come to dominate the group, others apparently being subjugated. Undoubtedly, such fish as come to dominate a group will take maximum rations as far as possible, while subjugated fish may or may not succeed in obtaining food.

The feature mentioned immediately above is also shown amongst females of the intermediate groups. In Table 6 it is shown that the initially smallest fish, F6, does not succeed in doubling its initial weight during the course of the experiment, while other fish easily achieve this result (F1, F2, F3 and F4). But the results of the competition for food

within the intermediate-fed groups are not as well marked among females as among males.

A comparison of Tables 5 and 6 will serve to show that the growth of intermediate-fed females closely follows that of the corresponding males. Table 7 shows that these females make rather more use of food for purposes of growth than did the corresponding males, 7·2 gm. and 7·7 gm. of food being required to produce 1·0 gm. of fish on the average, for females and males respectively. The difference is not as well marked, however, as in the cases of maximum-fed fish, where the corresponding food quantities are 10·5 gm. and 13·3 gm. But in both male and female groups there appears to be a marked increase in efficiency in the intermediate groups. This is difficult to explain, particularly in the case of the males, where a virtual mixture of maximum and minimum fish obtains. In order merely to balance the food bill of the intermediate group for the whole period, such a mixture of fish would need to consist of not more than two maximum fish to four minimum ones. Yet three or four of the fishes in the intermediate male group could be regarded as maximum fish as far as size is concerned, if rather poor ones. There thus appears some reason to believe that fish make more use of food for purposes of growth when it is present in medium quantity, than when it is particularly abundant. In work on digestion in the plaice, the results of which are unpublished as yet, it was found that food stays longer in the stomach if no second meal is given than it does if a second meal is taken. There may be relatively more complete absorption of food substances, therefore, when food is relatively more scarce. But it is not intended to stress unduly these differences of efficiency. It appears possible that although food from a previous meal was never observed in a box containing maximum-fed fish, yet particles of food left over from a meal might conceivably have been swept out of the box by a rising tide. Thus the question is left open to doubt on the results of the 1928 experiments alone. But it appears that much interesting information would be obtained by following up such suggestions as are here afforded by more conclusive experiments.

4. Conclusions Drawn from the Experiments of 1928.

(1) A male fish 16·0 cm. in length and 42 gm. in weight requires approximately 0·7–0·8 gm. of food per day in order to maintain its weight constant. For the major part of a growth-season of 176 days the maintenance ratio, i.e. the ratio of the quantity of food required daily to the body-weight, varied from 0·017 to 0·019, and may be taken as approximately constant at 0·018. During the months of December and January the ratio falls to 0·014.

(2) A female fish initially 13·4 cm. in length and 24·3 gm. in weight is able to increase its weight by 16·7 gm. when supplied with an average daily food ration of 0·6 gm. for 176 days. It is suggested that the true maintenance ration for such a fish lies between 0·4 gm. and 0·5 gm. per day. The maintenance ratio would then lie between 0·016 and 0·020.

(3) A fully feeding "mean" male fish initially 16·0 cm. in length and 38·8 gm. in weight increased its length by about 6·6 cm. and its weight by about 86 gm. during a period of 176 days from July 16th, 1928. It consumed 1129 gm. of food, an average of about 6·4 gm. per day for the period. It is estimated that roughly $\frac{1}{5}$th of the total quantity of food consumed was required for purposes of maintenance.

(4) A fully feeding "mean" female fish, initially 16·1 cm. in length and 43·3 gm. in weight, increased its length by 7·2 cm. and its weight by 107·9 gm. during a period of 176 days following July 16th, 1928. It appears probable that, since the total food taken was the same in quantity as that taken by the corresponding male fish, females make slightly better use of food for purposes of growth than do males.

(5) Considerable variation is shown in the growth taking place in different individuals in an environment holding an abundance of food. A difference of weight of 12–13 gm. between two fish at the commencement of the third season of growth may become amplified into a difference of 80–90 gm. by the end of this season.

(6) Fish supplied with daily rations of food intermediate in value between maximum and maintenance rations and about $\frac{1}{3}$rd the value of the former, appear to make better use of food for purposes of growth than do fish supplied with maximum rations. A comparison of efficiencies is given in Table 7 and probable explanations are afforded.

(7) In fully feeding fish, there appears to be no simple relation between the quantity of food consumed in a given period and the increase in weight.

II. OBJECTIONS TO THE FORM OF THE PRELIMINARY EXPERIMENTS.

Among the objections raised to the form of the preliminary experiments, there are several which are capable of being remedied or removed altogether. It was seen that, if a group of fish is supplied with food sufficient merely to satisfy maintenance requirements, the ration is not

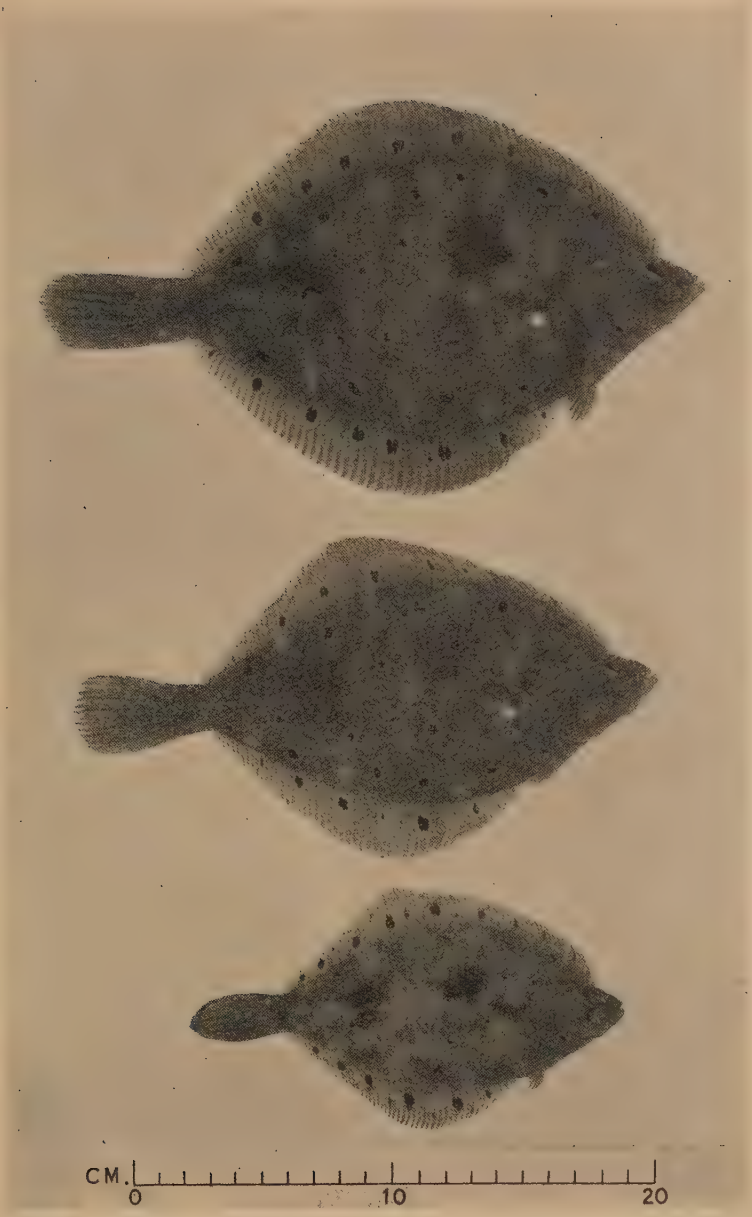

FIG. 4.—Female plaice photographed at the end of the preliminary experiments of 1928 [Cawsand]. The lengths are 16·8 cm. [bottom], 22·6 cm. [middle], and 25·7 cm. [top]. At the commencement of the experiments, the fish were of approximately equal size. The fish shown at the top of the figure was supplied with maximum rations and growth was unrestricted; that shown at the bottom was supplied with minimum rations sufficient to satisfy maintenance requirements without allowing of growth, while the middle fish consumed rations intermediate in quantity between the maximum and minimum ones, so that growth was slightly restricted.

Photograph by Mr. D. P. Wilson.

equally shared by all members of the group, certain fish coming to dominate the group and to overpower other members in the competition for food arising within the group. The dominant fish obtain more than the minimum ration intended for them, while the remaining fish do not receive sufficient food for purposes of maintenance, and decline, eventually to die. Larger fish may succeed in this competition where smaller fish fail, but size is not the sole factor determining success. It may be that the general condition of the fish is a factor. In any case it is not possible to ascertain which fish of such a group would fail to obtain food. Similarly in the case of groups of fish supplied with intermediate rations, certain individuals come to dominate the situation and obtain more than the average quantity of food, while certain others obtain a bare maintenance ration or little more. The only means of removing such undesirable conditions as are mentioned above, is to ensure that each fish receives the food intended for it, by keeping the members of the group completely separated from each other and by paying attention to individuals instead of to groups.

Another serious objection is the lack of any means of showing how the quantity of food necessary for purposes of maintenance varies with the size of the fish. During the course of the preliminary experiments, only a single set of minimum-fed fish of each sex was employed. It is desirable to show how the maintenance requirements of a fish vary during the course of the year, e.g. with differences of water temperature, but it is more important to ascertain how they vary for fish of sizes included between the initial and final sizes of the maximum-fed, fully growing fish during the same period, so as to be able to infer the requirements for maintenance purposes of the fully growing fish. The most practicable method in keeping with the nature of these experiments is to select individuals of various sizes passed through by growing fish during the course of the third season of growth and to endeavour to maintain the weight of these fish constant.

The presence of prawns in the sea-water pond at Pier Cellars proved to be a great source of annoyance. They were found regularly in the vicinity of the boxes, into which they were able to pass freely on account of the large size of mesh of the vertical faces. They were quite capable of removing food and preyed as far as possible on the rations presented to the fish. A special watch was kept for them, and they were removed as promptly and as often as they appeared, but obviously more than merely palliative measures were called for. As there also appeared some doubt as to whether or not food was ever swept out of the boxes, it seemed advisable to substitute a finer meshwork into the vertical faces of the boxes, a meshwork large enough to allow of an adequate circulation of water through the boxes and at the same time small enough to prevent the ingress of prawns and the egress of food particles.

III. MODIFICATION OF APPARATUS AND METHODS (1929).

Before commencing the experiments of 1929, alterations were made to the apparatus in order to eliminate the undesirable factors mentioned above. The $\frac{1}{2}$-in. mesh wire-netting windows of the boxes were removed and, in place of these, windows of perforated ebonite were substituted. Every box was provided with 4 windows each 12 in. $\times$ 12 in., and 4 more each 12 in. $\times$ 6 in., the perforations being $\frac{3}{16}$ in. in diameter and being set $\frac{5}{16}$ in. apart. The cavity of each box was divided into 4 equal compartments, by means of $\frac{1}{2}$-in. mesh, wire-netting partitions, provided with a 6-in. sill at the base to prevent the passage of food from one compartment of a box into another. Six boxes were thus transformed, so that accommodation was afforded for 24 fishes, and it was decided to continue the experiment with maximum-fed and minimum-fed fish only.*

During the course of the revised experiments, each fish was kept quite separated from its fellows, and received an independent food ration daily, or as nearly daily as was found practicable. In all cases, whether of minimum-fed or maximum-fed fish, food was placed in the box one day and the portion uneaten was weighed back before food was presented on the following day. It is important to observe carefully what portion of food is left over, particularly as a measure of the appetite of a fish on one day is by no means a certain measure for the following day, and since it is found that if food is left over it remains untouched subsequently until decomposition is complete.

Six separate fish of each sex formed minimum-fed groups and six of each sex maximum-fed groups, and the sizes of fish in each maximum-fed group were arranged to be as similar as was practicable. At the outset, minimum fish of as nearly as possible the same size as the maximum ones were employed. After a time the original minimum fish were in certain cases changed for larger fish, which were then kept on minimum rations. An attempt was thus made to ascertain the maintenance requirements of the freely growing fish, but difficulties were met with. A hasty substitution of fish meets with failure. For a variable period after capture, fish taken freshly from the sea refuse to feed, and lose weight to a considerable extent. It is better in practice to store such fish as are intended for later inclusion in the experiment as singly kept fish, in storage boxes in company with others of their kind. Newly captured fish which refuse to take food when kept alone, usually feed if kept with others of their kind.

It is very difficult to " match " freely growing fish with minimum fish. To do this a large number of " stored " fish of various sizes are required. There is considerable variation in the rate of growth of different individuals,

* This was the ruling of the officers of the Ministry of Agriculture and Fisheries.

and one cannot predict the increase in length of any fish a fortnight in advance, and a fortnight or more is needed to allow freshly-trawled fish to become settled. It was not practicable to store large numbers of fish alive at Pier Cellars, and hence little success was obtained in attempts to "match" such fish as were already included in the experiment. But some success was met with in the general aim of determining the maintenance requirements of fish of different sizes.

Weighings were made fortnightly as in the case of the preliminary experiments. But each individual fish was separately treated, the weighing apparatus and its water contents being reweighed for every single fish.

The experiments proper commenced on May 22nd, 1929, but for a period of days previously a large number of fishes were fed and observed regularly, all apparently unsound individuals being removed. From the remaining fish a selection based upon size requirement was made. The experiments were continued until December 12th, 1929, although severely interfered with during the gales which occurred early in the month. In most cases the data obtained during the last fortnight of the experiments were rejected, and in other cases damage to the apparatus resulted in the loss of fish so that data were not forthcoming.

It must be mentioned that great care was taken throughout the course of the experiment to keep the boxes free of epifauna and epiflora. The small size of the perforations in the ebonite windows made it imperative to keep the boxes clear of sedentary forms of life of all kinds, in order to maintain a circulation of water through the boxes.

IV. RESULTS OF THE CAWSAND EXPERIMENTS OF 1929.

1. Minimum Requirements and Maintenance.

Table 8 presents data obtained in experiments with a group of six male fish and a second group of six female fish, all kept separately and supplied with minimum rations from May 22nd to July 5th, a period of 44 consecutive days. During the period, four measurings and weighings were made, although in the table cited only the initial and final lengths and weights are given.

It is seen that, in males and females alike, the quantity of food necessary for purposes of maintenance varied in individuals of approximately the same size. Among the males, C4 lost 3·0 gm. in weight during the period when supplied with 0·43 gm., or 0·025 of the body-weight, of food per day. The fish C5, of smaller initial size, gained 3·0 gm. in weight when supplied with an almost identical average daily ration, while C3 maintained its weight constant (but increased its length) on 0·42 gm. of food per day. The mean fish increased its initial weight of 16·7 gm. by 1·7 gm. during 44 days, when supplied with 0·42 gm., or 0·024 of the body-weight, of food

per day. Among the females, A2, A5, and A6 show some variation, while the mean fish increased its initial weight by 0·5 gm., when supplied with an average daily ration for the period of 0·42 gm., or 0·019 of the body-weight of food. The increase in weight of the mean fish for the period is greater in the male group, so that the maintenance ratio is correspondingly smaller than the table indicates, approximating more closely to that of the female mean fish.

Data obtained from the experiments with the minimum-fed male fish C5a, C4a, and C6a from July 5th to November 28th, 1929, are shown in Tables 9, 10, and 11. The fish C5a lost weight during the early part of the experiment and never completely regained it, coming to within 1·0 gm. of its initial weight after 146 days. C4a and C6a also lost weight slightly during the early stages, but regained it and finally increased in weight slightly. The early loss of weight is due in all three cases to the fish's refusal to take food.

Inspection of Tables 9, 10 and 11 will serve to show that the average daily ration can hardly be fixed so that the weight of the fish remains constant. For example, C5a, for the periods ending August 30th, September 16th, and October 1st, when sea temperatures were fairly uniform, gained 2·0 gm. in 14 days, 1·5 gm. in 17 days, and 1·0 gm. in 15 days when taking 0·64 gm., 0·66 gm., and 0·80 gm. of food per day for respective periods (Table 9). Similar examples of the apparent variation in maintenance requirements are shown in Tables 10 and 11. It appears impossible to supply a fixed daily ration for purposes of maintenance so that the body-weight of the fish will be maintained constant.

In Table 12 is shown how the ratio of food taken per day/average body-weight varied for the fishes C5a, C4a, and C6a during the course of the experiment. The values of the ratio are calculated for each period and also cumulatively. Side by side with the data thus obtained are arranged the periodic increases in body-weight of the fish. The initial and final weights of a fish are shown on each side of the index of that fish. It is seen that for any fish, the slight increases in weight are disproportionate to the maintenance ratio, so that for the same fish a constant fraction of the body-weight of food per day will not serve to maintain the weight of the fish constant. Thus for C6a during the periods ending August 16th, August 30th, September 16th, and October 1st, when the maintenance ratio is 0·023, 0·023, 0·022, and 0·020, the increases in body-weight are 2·0, — 0·5, 1·0, and 0·5 gm. respectively. There is an appreciable fall in the value of the maintenance ratio during November, when the temperature of the sea-water of the pond falls. And it is seen also that the value of the maintenance ratio falls with increase in weight of the fish. Thus, the mean value of the ratio for the whole range of the experiments is 0·019 for C5a and 0·016 for C6a. This difference is rendered more conclusive

when it is observed that the larger fish was able to increase its initial weight on the food supplied.

The results of the experiments with the minimum-fed female fish A4a, A6a, and A3a respectively, are shown in Tables 13, 14, and 15. As was found to be the case in males, the relationship between the quantity of food supplied to any fish per period and the slight increase in weight of the fish for the corresponding period, varies from time to time [compare the daily ration per period and the weight increase per period for periods ending August 16th, August 30th, September 16th, and October 1st, A4a (Table 13), A6a (Table 14), and A3a (Table 15)]. The same fact is emphasised when the daily maintenance ration is expressed as a fraction of the body-weight, as in Table 16. The variations in the ratio of food supplied per day to the average body-weight of the fish are not considerable, except during the early stages of the experiments when food was refused and for A3a during the period ending October 15th, but the differences in the increases in body-weight are well marked, e.g. during the periods between August 2nd and October 1st for the fish A6a.

The results shown in Table 16 do not show conclusively any appreciable fall in the value of the maintenance ratio with increased size of fish, but the fall in value of this ratio during November is admirably shown. Table 17 is compiled from Tables 9–16, to show as nearly as is possible, and for the whole range of the experiments, how the maintenance requirements of fish of various sizes and of both sexes varied. It is seen that for fish of approximately the same size there is no appreciable differences of these requirements for the two sexes. Table 18 shows how the maintenance requirements of fish of various sizes varied for a relatively shorter period towards the end of the experiments and particularly how the value of the maintenance ratio diminishes as the sea temperature falls during November. It must not be overlooked that the increases in weight shown on the right are for a period of 14 days; those on the left for a period of 43 days.

A striking feature concerning growth in length is observed in the results of these experiments. Table 9 shows that for the whole range of the experiment with C5a, there was a loss of weight of 1·0 gm., although an increase in length of 0·4 cm. For C6a from July 12th to October 1st, the weight decreased by 0·5 gm., while the length increased by 0·5 cm. (Table 11). For the females A4a and A6a, during the course of the experiments to October 1st and September 16th respectively, the weights decreased by 1·0 gm., while the lengths increased by 0·5 cm., in each case (Tables 13 and 14). It appears, therefore, that when loss of weight occurs, it is not usually accompanied by decrease in length, and that when weight is regained there occurs contemporarily an increase in length. The effect

of this phenomenon is to lower the general condition of the fish as indicated by the weight/length^3 ratio.* Thus, taking the female fish A4a, which lost 7·0 gm. in weight during the period of 14 days ending July 19th (Table 13), the initial value of this ratio was 0·010. By July 19th the value had fallen to 0·008 and the initial value was never regained during the course of the experiment, the values for October 15th and November 27th being 0·009. Similarly, the female fish A6a incurred a loss of weight of 3·5 gm. during the first fortnight of the experiment, and did not recover the initial value of the weight/length^3 ratio (0·010) until November 27th, although consistently gaining in weight after the first loss. The male fish C5a did not show a value for the ratio as high as the initial one of 0·010 during the whole range of the experiment.

2. Maximum Requirements and Growth.

The results of the experiments with maximum-fed fish are varied. There was shown considerable variation in the rate of growth of different individuals, and in several cases failure was met with, where what appear to be abnormal fish made no appreciable growth whatsoever. The final results obtained with the six fishes of each sex are shown in Table 19. The total increases in size are indicated side by side with the total quantities of food consumed and the average daily rations for the whole period are shown. It is important to realise that references to food quantities are also references to appetite shown by the fish, since throughout the experiment, food left over from a meal was consistently " weighed back " before the following meal was presented.

It will be agreed that certain fish can be excluded from consideration (Table 19). A 2-year old fish which increases its weight by 5·0 gm. during 117 days, or 7·0 gm. during 175 days, is not progressing satisfactorily. The fish B3 showed signs of inability to co-ordinate movements, swimming inverted and settling down on the vertical faces of the box as if on the floor of it. Accordingly, such fish as D6, B3, and B5 will be disregarded. In the D group, D3 made most progress, and will receive special consideration as showing the maximum growth for the experiments. The fishes D2 and D4 will be compared with D3. In the B group, B1 and B4 show the maximum growth for the experiment, and B2, B5a, and B6 will be compared with them.

It is seen from Table 19 that the average quantity of food consumed per day for the whole range of the experiments is about 3·3 gm. for the fish showing maximum growth, males and females alike. The weight of

* The constant K, which is a measure of the general condition of a fish, is usually defined as $\frac{W \times 100.}{L^3}$. Here it is taken as $\frac{W.}{L^3}$.

food necessary to produce 1·0 gm. of fish, estimated for the whole range of the experiment works out as follows :

D3 (♂)—6·6 gm.
B1 (♀)—7·6 gm.
B4 (♀)—7·2 gm.

In these instances, therefore, the efficiency of the male appears to be greater than that of the female, contrary to the findings during the preliminary experiments. For all fish, good, bad and indifferent, and for the whole range of the experiment, 11·6 gm. of food produces 1·0 gm. of mean male fish and 13·9 gm. of food produces 1·0 gm. of mean female fish, so that here again the male appears to possess greater efficiency.

The data concerning the male fish D3 are shown in Table 20. It is seen that increases in body-weight for any specified period reach a maximum during September. This is also shown in the acceleration curve (i.e. a curve showing the increase or decrease in value of periodic increments from time to time, or in other words, showing the rate of change of growth) of body-weight in Fig. 5. The quantity of food consumed per day is a maximum during early October, but is near its maximum value during September, as is also shown in Fig. 5. The general condition of the fish as indicated by the value of the weight/length^3 ratio, is at its best when the appetite of the fish is greatest and when maximum growth is taking place, as would perhaps be anticipated.

Periodic increases in body-weight are shown in Fig. 5 in the form of an acceleration curve, while the growth curve of weight is shown above. The latter is seen to be a smooth, slightly asymmetrical, sigmoid curve of typical form. The steepest part occurs during mid-September, and the point of inflection for the season is not reached at the close of the experiment. No attempt has been made to find the equation to this single-fish curve.

It is also seen from Table 20 that the efficiency of the fish, as expressed by the quantity of food necessary to produce 1·0 gm. of fish, varies from period to period during the course of the experiment. The efficiency is greatest during September, when the weight increments of growth are greatest, when 4·8 gm. to 4·9 gm. of food are required. Thus it is indicated that factors other than merely the quantity of food consumed operate. There is a period of increased efficiency during early July, and it is worthy of remark that two peaks occur on the temperature graph for the season which closely correspond with these periods. The efficiency of the fish estimated to any date varies between 5·6 gm. and 8·8 gm. of food per gm. of fish over the initial weight.

Growth in length of the male fish D3 appears to be a linear function of time, at least for the major part of the experiment, as is shown by

inspection of the figures given for length in Table 20. Plotting length against time, we obtain the curve shown in Fig. 9. Until the middle of October, the points plotted arrange themselves evenly and fairly closely about a perfectly straight line.

The results of the experiment with the male fish D2, which are shown in

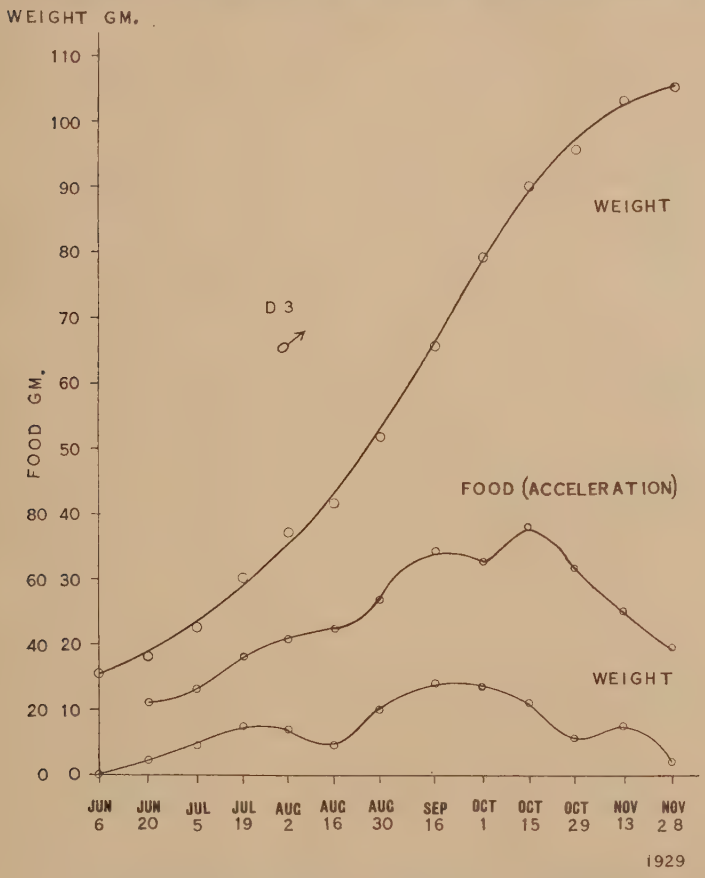

FIG. 5.—Growth curve of weight of the male plaice D3 together with acceleration curves of periodic body-weight and food-weight increments. (Cawsand, 1929.)

Table 21, are distinctly interesting. As in the case of D3, the quantity of food consumed per day reaches a maximum during late September, when the condition of the fish, as indicated by the weight/length^3 ratio, was at its best. But growth in weight is not at all regular, and the efficiency of the fish varies in a striking manner (see Column 8, Table 21). During July

there is an acceleration of growth in weight (Table 21 and also Fig. 6), then during August a retardation, followed by a great acceleration during the early part of September. There is here a strong suggestion that growth in weight is connected with temperature, the two accelerations in growth coinciding with peaks on the temperature graph (Fig. 11), the retardation with a fall in temperature during August. The curve of growth in weight (Fig. 6) suggests that this fall in temperature interfered in some way with the metabolic processes of the fish and that growth was slowed down

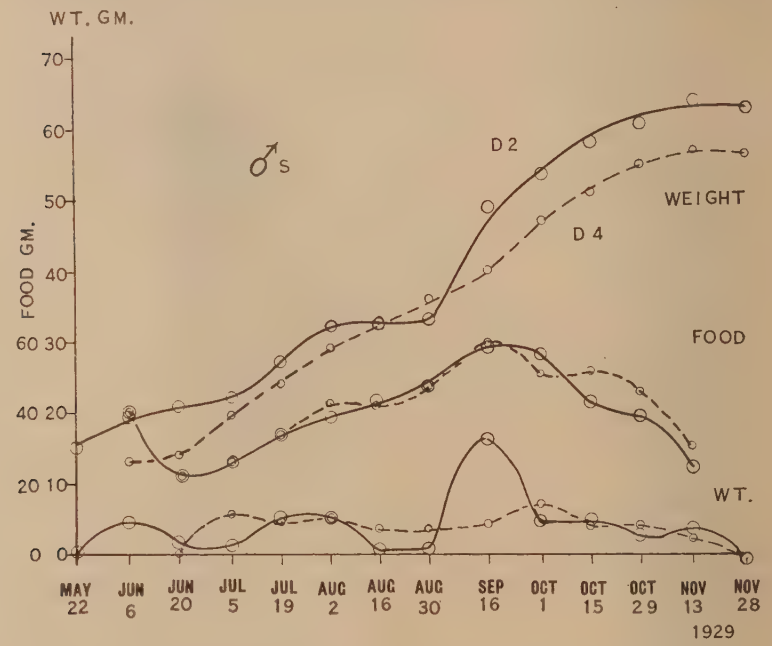

Fig. 6.—Curves of weight and acceleration curves of body-weight and food increments for the male fishes D2 and D4. (Cawsand, 1929.)

until the great acceleration during September, which proved too late to produce a typical result such as was shown by the fish D3. The efficiency of the fish D2 during early September, when 3·6 gm. of food produced 1·0 gm. of fish, is truly remarkable. It greatly transcends that of D3 for any period during the year, although the appetite for food after the first fortnight was never as great as in the latter fish.

The growth in weight of D4 follows that of D2 fairly closely. The total increase in weight for the whole period (Table 22) is comparable with that shown by D2, as is also the total quantity of food taken, and therefore the average efficiency of the fish. But in the case of D4, there

is no phenomenal acceleration of growth in weight. Instead, growth is more regular, but attains a maximum during late September. There is shown a slight retardation of growth during August, which is comparable with the more marked one shown by D2 (Fig. 6).

A consideration of the growth of the male plaice D3, D2 and D4 shows

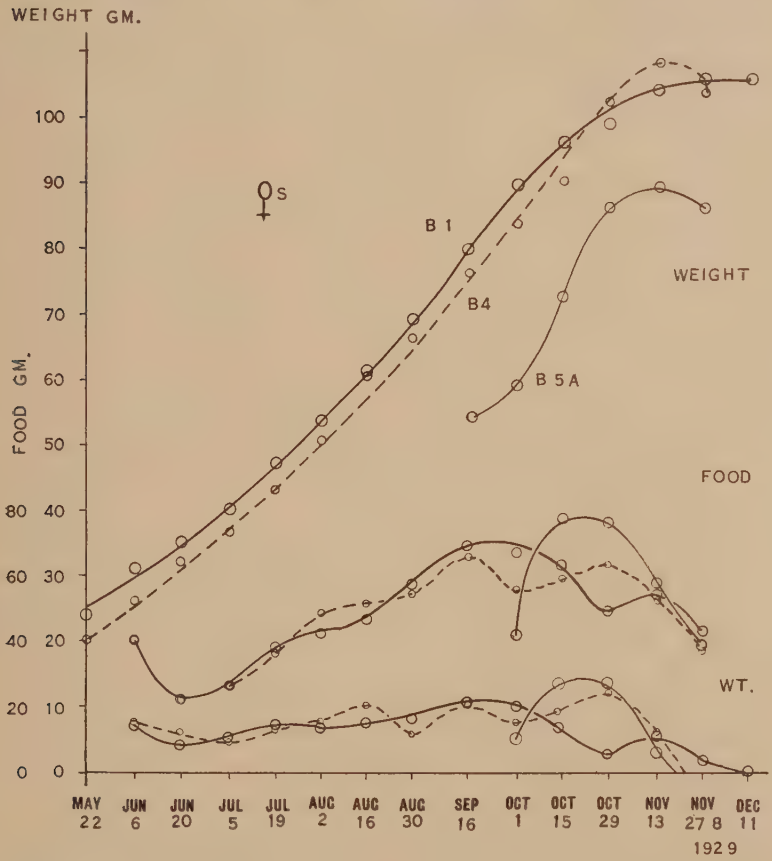

FIG. 7.—Growth curves of weight and acceleration curves of body-weight and food-weight for the female fishes B1, B4 and B5a. (Cawsand, 1929.)

then that September is the month of greatest appetite and maximum growth. It is also apparent that temperature influences growth and that, given a suitably high temperature, fish which have suffered a retardation of growth are able to become increasedly efficient in an attempt to compensate for the growth inhibition.

It has been observed above that the growth taking place in female

fish closely follows that in the male fish, for individuals showing the maximum growth for the experiments. The females B1 and B4 show a very close agreement and can be considered side by side. Table 23 presents the data concerning B1, Table 24 that concerning B4. In the case of B1, the maximum growth in weight occurs during September, when over 20 gm. are added to the body-weight. In the case of B4, growth is accelerated during September, but attains a maximum during October, when over 18·5 gm. are added to the body-weight. In each case maximum growth is accompanied by maximum appetite. In the case of B1, the

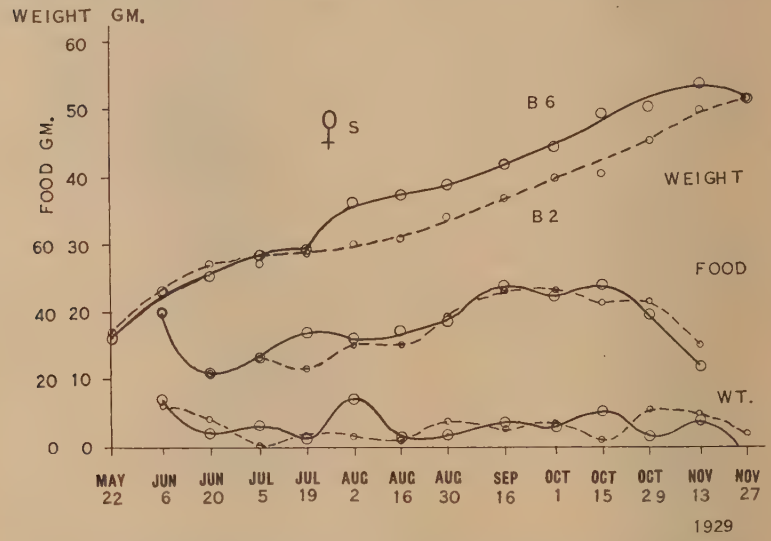

Fig. 8.—Growth and acceleration curves of body-weight and food-weight for the females B6 and B2. (Cawsand, 1929.)

weight/length^3 ratio is also a maximum during the period of maximum growth. As was found to be the case in the male fish D3, the efficiency of the females B1 and B4 varies from period to period during the growth season, but there is no marked increase in efficiency when growth and appetite attain maximal values.

The curves of growth in weight of the females B1 and B4, like that of the male D3, are sigmoidal in form (Fig. 7). During the first half of the experiment the curves follow each other closely, but differences are shown towards the end of the period. The point of inflexion of the curve for B4 is reached in mid-November, while that of the curve for B1 may be considered as reached during early December. The acceleration curves of food and body-weight increments for the fish B1 and B4, shown in Fig. 7, suggest that there is some connexion relating them,

despite the periodic variations in efficiency indicated from period to period.

The curves of growth in length of the fishes B1 and B4 are shown in Fig. 9, side by side with the corresponding curve for the male D3. It is seen that, as in the case of the male, this curve is a straight line one for the early part of the experiment in each case, and that the curves for the female

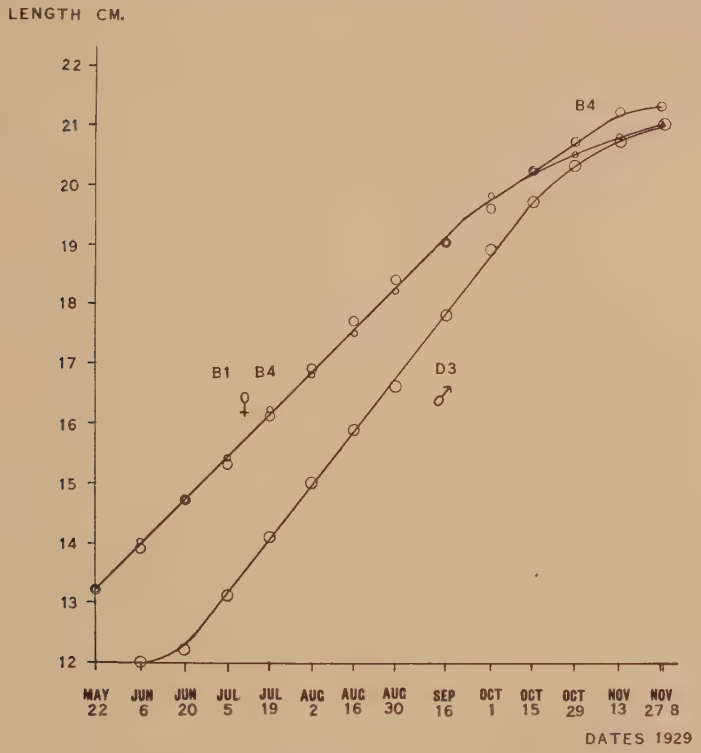

FIG. 9.—Growth curves of length for the male D3 and the females B1 and B4. (Cawsand, 1929.)

are very similar. The slopes of the B1, B4 curves are less steep than that for the D3 one. Even during the latter parts of the experiments, the relationship between length and time appears to be a linear one, a straight line with a lesser slope being obtained.

On September 17th the female fish B5a was substituted for an unsatisfactory fish B5, and the data concerning the substituted fish are shown in Table 25. The curve of growth of this fish, together with acceleration curves for body-weight increase, and food increments is shown in Fig. 7.

This fish is especially interesting in that it shows a remarkable acceleration of growth in weight during October, together with a greatly increased appetite for food and a rapid improvement of the general condition as indicated by the weight/length^3 ratio. The body-weight of this fish was increased during October by more than 27 gm., and during this month it consumed at least 1·0 gm. of food per day more than the fishes B1 and B4, at the same time showing an increased efficiency over these fish. The point of inflexion of its growth curve was reached very shortly after the

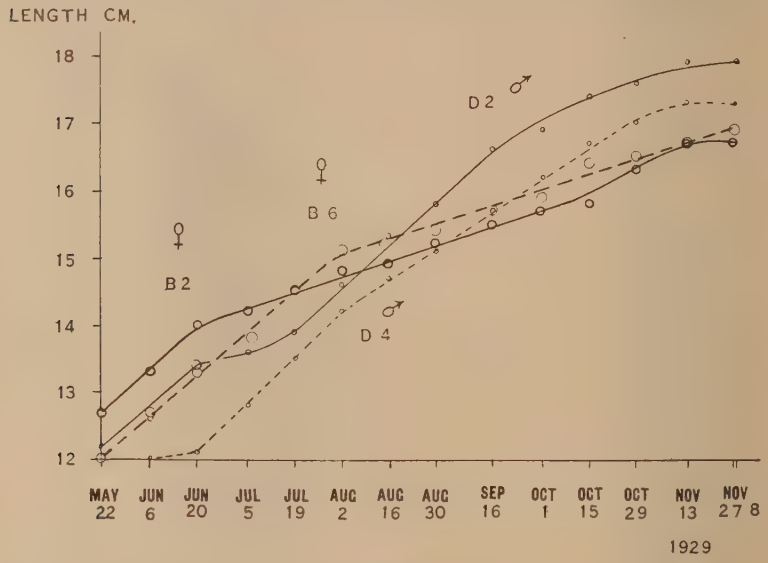

Fig. 10.—Growth curves of length for the males D2 and D4 and the females B2 and B6. (Cawsand, 1929.)

period of maximum growth and towards the end of the experiment it was losing weight.

Growth occurring in the fishes B2 and B6 during the earliest periods of the experiments compares closely with that occurring in the fishes B1 and B4. They gained substantially in both length and weight (Tables 26 and 27), and improved in general condition. But both received setbacks which inhibited growth and from these never completely recovered, although B2 made attempts during late August and September, B6 during late July and early October. B6 maintained a good general condition, as measured by the weight/length^3 ratio, throughout. The growth curves and acceleration curves of body-weight and food increments are shown in Fig, 8.

The growth curves of length for the females B2 and B6 are shown in

Fig. 10 along with those for the males D2 and D4. Taking B2 first, it is seen that inhibition of growth in length took place during late June, after a short period of what appears to have been typical growth. This inhibition of growth in length is paralleled by a corresponding inhibition

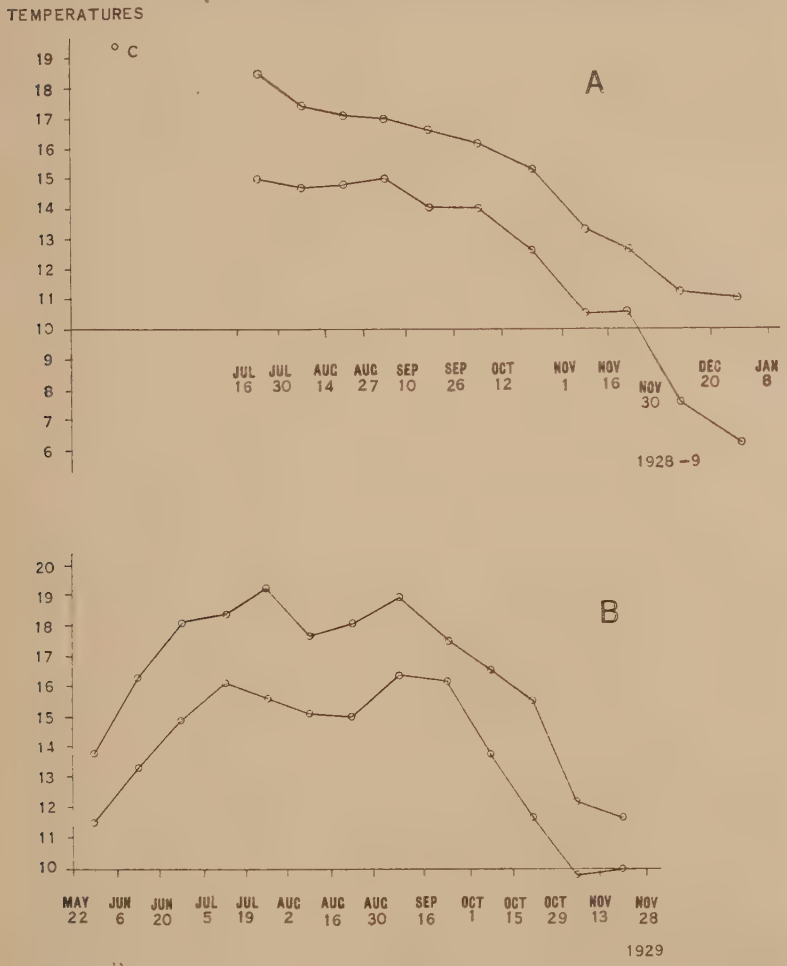

FIG. 11.—Graph of maximum and minimum temperature at Cawsand. [A] 1928–29; [B] 1929.

shown by the male D2. A difference is seen to follow the inhibition. Whereas in B2 the inhibition is permanent, the relationship between growth in length and time continuing to be a linear one yet a different one as shown by the change in the slope of the curve (Fig. 10), that in

D2 is temporary, the old linear relationship being continued at least until mid-September. These characteristics are also shown by the curves of growth in weight, D2—Fig. 6, and B2 Fig. 8. The inhibition of growth in weight shown by the male fish D2 during the month of August and attributable to a fall in temperature is not accompanied by inhibition of growth in length, the same linear relationship between growth in length and time obtaining throughout, from mid-July to mid-September. The case of the female B6 is different, however. Growth in both length and weight is typical until August, after which growth inhibition occurs in both respects (Figs. 10 and 8). This inhibition may be attributed to fall in temperature of the sea-water in the pond. It thus appears that growth in both length and weight is influenced by temperature.

A temperature graph for the 1929 experiments is shown in Fig. 11 (B). A similar graph for the preliminary experiments of 1928 is also shown (A).

3. Conclusions Drawn from the Experiments of 1929 (Cawsand).

(1) A mean male fish 12·7 cm. in length and 16·7 gm. in weight was able to maintain itself and also to increase its weight by 1·7 gm. during 44 days from May 22nd, 1929, on an average daily ration of 0·43 gm. of Mytilus flesh. The maintenance ratio, i.e. the ratio of food consumed per day/average body-weight, approximates to 0·025, but it is suggested that this value is high as is shown by the slight increase in weight. A more accurate value would approximate more closely to the value obtained for the mean female fish (*vide infra*).

(2) A mean female fish 13·6 cm. in length and 22·1 gm. in weight gained 0·5 gm. in weight during the period stated above, when it consumed an average daily ration of 0·42 gm. of food, or 0·019 its average body-weight.

(3) The average daily ration of food required to maintain the weight of any male or female fish constant appears to vary from period to period during the course of the experiments.

(4) The ratio of food required per day for purposes of maintenance/average body-weight of a fish appears to vary in different fish. In general it tends to fall in value with increase in size of the fish.

(5) The maintenance requirements of any fish appear to diminish appreciably during late October and November, and the fall is synchronous with fall in temperature of the sea-water.

(6) Loss of weight shown by any fish is not generally accompanied by decrease in length, and the regaining of weight is accompanied by

increase in length. The results of such loss of weight may be shown in the lowered general condition of the fish, as indicated by the weight/length^3 ratio, for months following the loss, even though a slight food allowance is available for purposes of growth.

(7) The growth curve of weight of fully-fed male and female fish is sigmoidal and asymmetrical. The maximum acceleration of growth in weight appears to occur during late September, when appetite for food is also a maximum. The efficiency of a fish, as measured by the quantity of food required to produce 1·0 gm. of fish, varies from time to time. In the male fish the greatest efficiency obtains when growth and appetite attain maximal values.

(8) Growth in length of male and female fish alike is an arithmetical function of time. The relationship holds good for the period of most rapid growth during the season.

(9) Growth inhibition may be followed by a strikingly increased acceleration of growth, when the fish displays a greatly enhanced efficiency. Appetite may be increased tremendously at the same time.

(10) Temperature appears to influence growth directly. Thus a fall in temperature may induce an inhibition of growth in both length and weight. If an increase in temperature follows the inhibition is overcome, other things being equal, and growth acceleration occurs. But such inhibition may also be rendered permanent for the growth season considered.

V. RESULTS OF THE LYMPSTONE EXPERIMENTS OF 1929.

1. Introduction.

A series of experiments similar to the revised experiments of Cawsand was carried out at the Ministry of Agriculture and Fisheries Mussel Tanks at Lympstone in the estuary of the River Exe, commencing on June 24th, 1929, under the care of Mr. H. Lees, Tank Superintendent. The boxes used were made at Cawsand and were identical with those used in the revised Cawsand experiments. They were suspended in the large tank A. The methods employed were precisely the same as those employed in the Cawsand experiments, and the same precautions were taken in weighing back food left over from any meal. Mr. Lees also kept temperature records of the water daily. The data obtained from the experiments were handed over to me by Dr. E. S. Russell in order that a comparison could be afforded of the two series of parallel investigations.

2. Minimum Requirements and Maintenance.

The results of the experiments on minimum requirements and maintenance with the male fishes, L1, L4, L3 and L2 are shown in Tables 28, 29, 30 and 31. It is seen that in all cases, excepting that of L4b, there is a slight loss of weight during the early stages of the experiments, but that in all cases the lost weight was regained and slight increases of weight occur.

The average quantity of food taken per day by the male fish L1 during periods included between September 16th and November 25th is seen to have been fairly constant (Table 28), but the weight of the fish varies during successive periods. For the periods ending September 30th, October 14th and November 11th approximately 0·95 gm. of food allows of an increase in weight. For the periods ending October 28th and November 25th, 0·90 gm. of food per day results in a slight loss of weight. Thus, between 0·90 gm. and 0·95 gm. per day appears to be a measure of the maintenance requirements of this fish. For the whole range of the experiment to November 25th (and it is not until slightly later than this that both maximum and minimum temperatures fall below 10° C.), 0·90 gm. of food per day allows of an increase over the initial weight of 3·8 gm. during 95 days. The average weight of the fish has been 52·2 gm.

L4a, a fish of 84·7 gm. weight, loses weight on 0·85 gm. of food per day, which, judging according to the requirements of L1, seems an inadequate daily ration. However, for a period of 14 days, 0·88 gm. of food per day allows of a slight increase in weight (Table 29). The slightly smaller fish, L4b, to November 25th, loses weight if the daily ration falls below 1·0 gm. (and in one case when it is as high as 1·30 gm.). For the whole range of the experiment to November 25th, an average daily ration of 1·11 gm. of food allows of a slight increase in weight (1·8 gm.).

L3, with an initial weight of 105·3 gm., loses weight if less than 1·30 gm. of food is taken daily per any fortnightly period, and gains weight if more than 1·38 gm. is taken (Table 30). For the whole period to November 25th, a daily ration of 1·32 gm. of food allows of an increase in weight of 4·1 gm. During this period, 78 days, the fish has taken approximately its own weight of Mytilus flesh.

L2 suffers loss in weight when the daily ration for any fortnightly period to November 25th is less than 1·30 gm., and gains in weight if this ration is over 1·66 gm. Taking the whole range to November 25th, 1·44 gm. of food per day results in a loss of 0·5 gm. from the initial weight of 129·9 gm. (Table 31).

In Table 32 are shown the results of two shorter range experiments, which do not appear to conform to those of the longer range experiments mentioned above. The maintenance requirements of L1a appear

especially to be relatively low ones, those of L2a high ones taking the loss of weight into account (cp. with L1, Table 28).

In Table 33, the daily rations supplied to the male fishes L1, L4b, L3 and L2 are shown as fractions of the average body-weight. The order presented is one of increasing body-weight from left to right, initial and final weights being shown on each side of the fish index. The maintenance ratio is shown for each fortnightly period and also cumulatively from the commencement of the experiment. For L1 the value of the ratio for periods from August 19th to November 25th varies between 0·015 and 0·019. For L4b the corresponding variation is from 0·010 to 0·018, for L3 from 0·010 to 0·015 and for L2 from 0·009 to 0·014. Thus the range tends to become lowered with increase in size of the fish. If the whole period to November 25th be taken, the values of the ratio for these fish, taken in the order presented above, are 0·017, 0·015, 0·012 and 0·011. The accuracy of the last figure is open to some doubt being slightly beyond the accuracy of the weighings, but the argument is not thereby robbed of its force, especially as the values given are all subject to the same errors. Thus there appears reasonable proof of the fall in value of the maintenance ratio with increase in size of the fish. The same conclusion is reached if the values for the daily rations, estimated to November 25th, are plotted against the body-weight of corresponding fish.

For female fish the results are similar to those of males. Neglecting for the moment results obtained after November 25th, L5 shows a loss in weight if the average daily ration falls below 0·81 gm. (except in one instance), and an increase in weight if it is 0·81 gm. or over (Table 34). To November 25th 0·88gm. of food per day results in an increase over the initial weight (62·8 gm.) of 4·1 gm. L6 (initial weight, 71·2 gm.) loses weight when the daily ration falls as low as 1·1 gm. and gains weight if it is over 1·2 gm., except during late November. To November 25th an average daily ration of 1·19 gm. results in a slight loss of weight (1·1 gm.) (Table 35). L7 (initial weight, 104·8 gm.) suffers loss of weight if an average quantity of food less than 1·4 gm. per day is taken, except during November, and to November 25th loses 4·2 gm. in weight after having consumed an average daily food ration of 1·32 gm. (Table 36). L8a (initial weight, 102·0 gm.) requires approximately 1·3 gm. of food per day, while L8b (initial weight, 114·0 gm.) loses 0·4 gm. in weight on an average of 1·51 gm. of food per day (Table 37).

The average daily rations of the female fishes mentioned above are shown as fractions of the average body-weight in Table 38. Estimates are shown for each fortnightly period, and also cumulatively from the commencement of the experiments. The fall in value of the maintenance ratio with increasing size of fish is not as well marked as in the case of males, but is shown all the same. To November 25th the values, taken

for increasing sizes (left to right, Table 38) are 0·014, 0·017, 0·013 and 0·013. The fishes L5 and L6 are of approximately the same size and these results show the likelihood of considerable individual variations in maintenance requirements.

The results of shorter range experiments are presented in Table 39. The smallest female, like the smallest male, requires a relatively high average daily food ration. This is also shown in Table 40, where it is

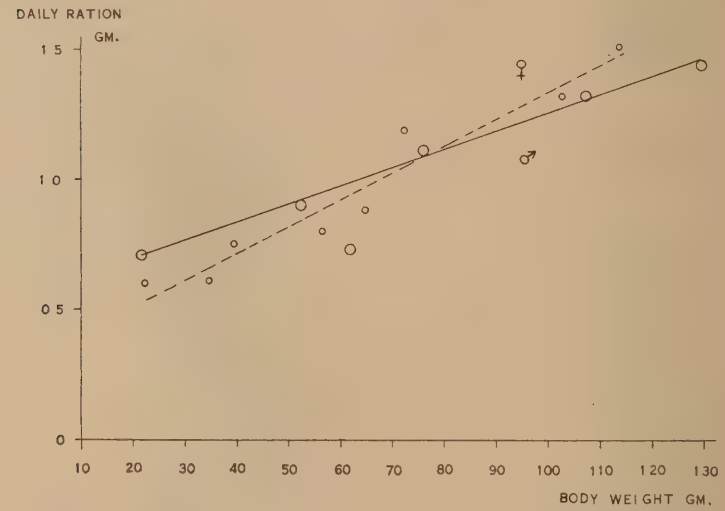

Fig. 12.—Graph showing the variation in maintenance requirements with size of fish. The abscissæ represent body-weights, the ordinates values of daily rations for purposes of maintenance. (Lympstone.)

also seen that despite considerable variations, there is a decrease in value of the maintenance ratio in larger fish.

Fig. 12 attempts to show graphically the relationship between the size of various fish and their maintenance requirements. These values are taken from preceding tables, and represent averages to November 25th except in the lower part of the graph where values from Tables 32 and 39 are employed. The points plotted are :

[♀] (gm.)	L6a	L5a	L8a	L7a	L5	L6	L7	L8a	L8b
Average Body-Wt.	22·3	34·6	39·4	56·5	64·8	71·7	102·7	103·2	113·8
Average Daily Ration	0·60	0·61	0·75	0·80	0·88	1·19	1·32	1·32	1·51

[♂] (gm.)	L2a	L1a	L1	L4b	L3	L2
Average Body-Wt.	21·6	62·0	52·2	76·0	107·3	129·6
Average Daily Ration	0·71	0·73	0·90	1·11	1·32	1·44

It is seen that the value of the ratio probably falls off with increasing size of fish, more rapidly in males than in females.

3. Maximum Requirements and Growth.

The final results of the experiments on maximum requirements and growth at Lympstone are summarised in Table 41. As in the Cawsand experiments, considerable variation is shown in the growth of different individuals. One male (L11) made great progress, while two females made very satisfactory efforts to rival this male. The average quantities of food taken per day vary considerably, and for the fish displaying the most rapid growth-rate the quantity is slightly more than 6 gm. per day. It is surprising to note that this average daily ration is almost twice as great as that consumed by the most actively growing fishes at Cawsand (3·3 gm. per day). Growth was correspondingly greater at Lympstone, but the efficiency of the Cawsand fish was slightly greater, in male and female alike. At Lympstone the male again appeared to be rather more efficient than the female.

Data concerning the experiment with the male fish L11 are shown in Table 42. Maximum growth takes place during August although the increments added to the weight of the fish during September and October are almost as great as those for August.* This is also shown in the acceleration curve for body-weight increase increments (Fig. 13). The general condition of the fish, as shown by the weight/length^3 ratio, is best during October and November, and better than was found in any instance at Cawsand. The appetite of the fish is greatest during late September and early October, when the efficiency of the fish is near its maximum value, and the maximum daily ration on the average for the experiment is 7·5 gm. It is noteworthy that this fish took from 7·0 gm. to 7·5 gm. of food per day consistently from July 22nd to October 14th, i.e. there are no peaks on an acceleration curve of food increments such as are found on the corresponding curve for increments of increase in body-weight. The efficiency of the fish varies considerably from time to time, which suggests that there is no continuous direct relationship between the quantity of food consumed and growth in weight. Growth in length appears to follow a linear relationship with time (Fig. 14), and it appears strange that the curve for growth in weight is representable as a straight line curve to November 11th, when inhibition takes place (Fig. 13).

The male fish L10 barely doubled its initial weight during 98 days (Table 43), consuming 9 times the average body-weight for the whole period of food. Maximum growth occurred during July, but the best general condition obtained during late August, when the appetite was greatest and the efficiency low. The curve of growth in weight is shown in Fig. 13. And L9 did not succeed in doubling its initial weight although it consumed 8½ times its average body-weight for the whole range of the

* The increase in weight for July 8th is neglected.

experiment (Table 44). Maximum growth obtained in this fish during late July and early August and here, when appetite was a maximum, efficiency was very low. The growth curve of weight is shown in Fig. 13. The male fish L12 displayed a greater average growth-rate (Table 45).

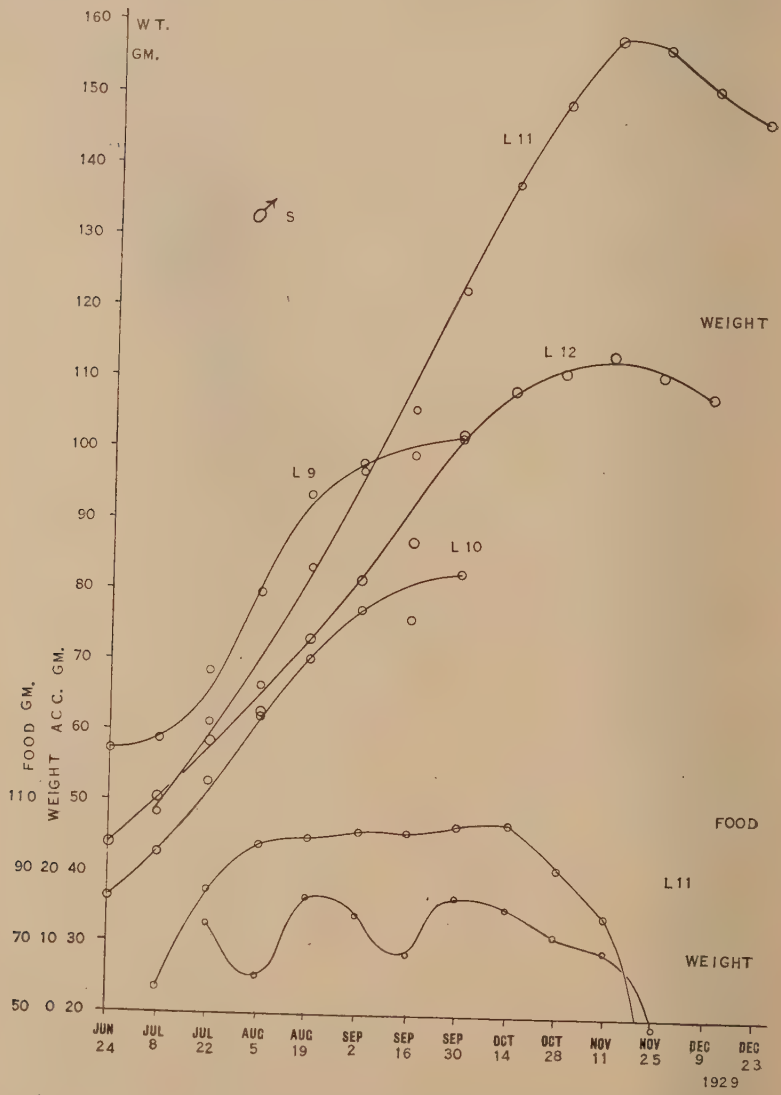

Fig. 13.—Growth curves of weight for the males L9, L10, L11 and L12, together with acceleration curves of food and body-weights. (Lympstone.)

Maximum growth in weight occurred during September, although growth was also rapid during August. The efficiency of the fish varied considerably, and was high during early August and late September. During the latter period the general condition was at its best. The curve of growth is shown in Fig. 13.

Among the females, L15 progressed well. The final weight at the point

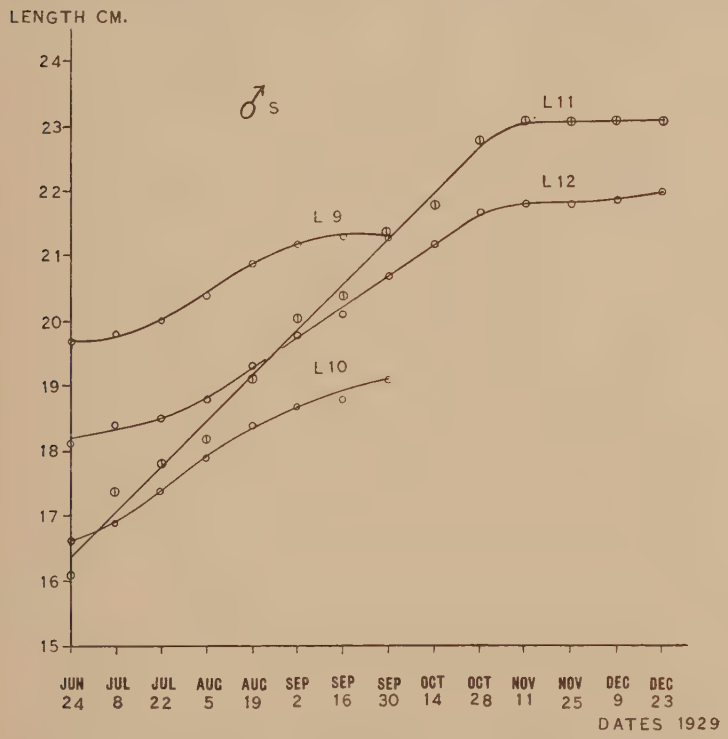

Fig. 14.—Growth curves of length for the males L9, L10, L11 and L12. (Lympstone.)

of inflexion of the growth curve was $4\frac{1}{2}$ times the initial weight, and approximately 10·7 times the body-weight of food had been taken. Growth in weight was most rapid during August, but almost as rapid during September and October (Table 46). Appetite was greatest during late September and early October, when efficiency was highest.

The efficiency of L16 was consistently high, its lowest during growth being 10·2 gm. of food per gm. of fish, and the appetite was similarly very great. No other fish took as much as 8·9 gm. of food per day, which quantity this fish consumed consistently for a month, from September 16th

to October 14th, during which period the body-weight was increased by 38·6 gm., the greatest increase recorded for any two consecutive fortnightly periods. During this period of rapid growth the highest value for the

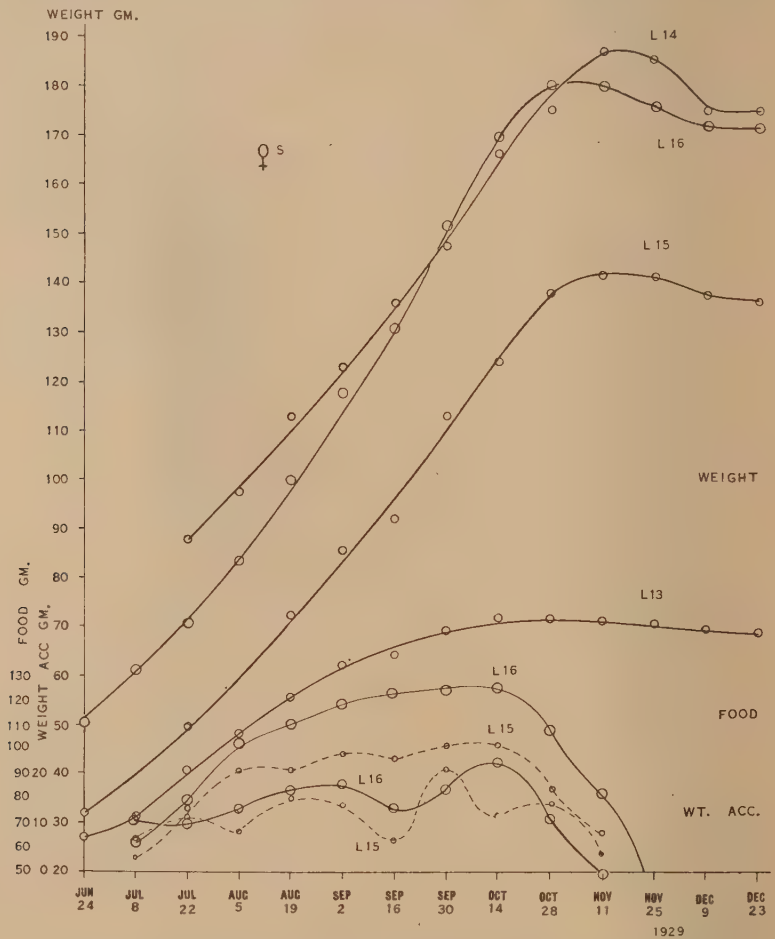

FIG. 15.—Growth curves of weight for the females L13, L14, L15 and L16, together with acceleration curves of food and body-weights for L15 and L16. (Lympstone).

weight/length^3 ratio recorded for these experiments was reached (Table 47). The growth curve of weight is shown in Fig. 15.

The growth-rate of the female fish L13 was very slow, an increase in weight of 9·5 gm. during July being the greatest for any fortnightly period during the course of the experiment. The efficiency of the fish was

low, especially after July, and the general condition ceased to improve. It seems probable that inhibition of growth took place during August (Table 48 and Fig. 15).

The female L14 is probably a 3-year old fish, and it is interesting to compare it with L16. For both fishes alike, maximum growth for any

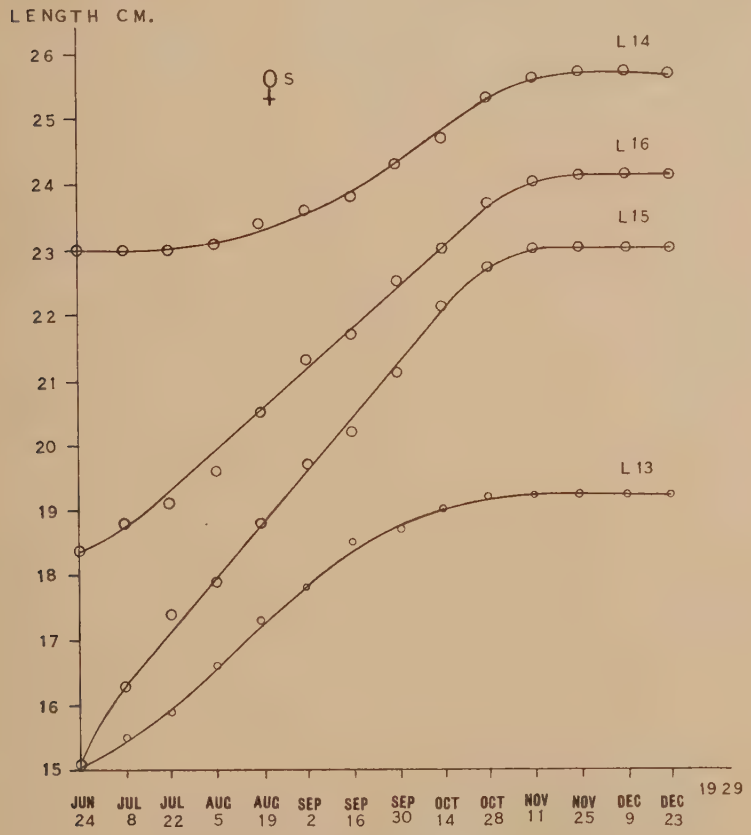

Fig. 16.—Growth curves of length for the females L13, L14, L15 and L16. (Lympstone.)

two consecutive periods occurs during late September and early October, when appetite is greatest, efficiency relatively high and the general condition at its best (Tables 49 and 47). The smaller fish took more food, however, and eventually reached to within 9 gm. of the final weight of the larger.

A characteristic feature of growth of the Lympstone fishes is the

abruptness of a retardation of the process and the loss in weight incurred immediately after growth inhibition, during late November and December. Appetite diminishes very considerably when the maximum temperature approaches 10° C. and is lost altogether when this temperature sinks below 10° C. During the growth season there does not appear to be any simple relation between the quantity of food taken per any fortnightly period and the increment of growth in weight for the period. The acceleration curve for food taken is an unbroken one for several consecutive periods, while that of added increments of weight is broken and shows two or three

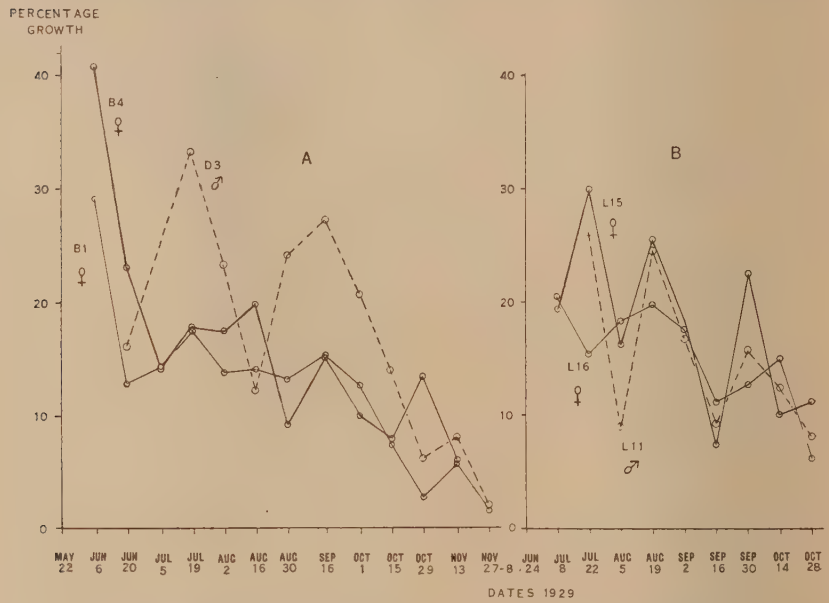

FIG. 17.—Graph showing percentage growth during specified periods for certain maximum fed fish. [A] B1 and B4 (♀'s) and D3 (♂), Cawsand, [B], L11 and L16 (♀'s), and L15 (♂), Lympstone.

peaks (Fig. 13 ♂ and Fig. 15 ♀). The efficiency of any fish appears to vary considerably from period to period.

Growth in length of maximum-fed male and female fishes is shown graphically in Figs. 14 and 16, and it is readily seen that considerable variation occurs in different individuals, especially in the case of males. In the cases of fish of either sex showing any considerable growth, a straight line curve is obtained from July to October. Table 50 (B) and Fig. 17 (B) attempts to show how the percentage growth in weight varies from period to period in both sexes. A coincidence is seen in the fluctuations in percentage growth of the male L11 and the female L15, which

suggests that a factor which operates to modify the growth-rate from period to period influences both sexes. From August 5th to the close of the experiments, L16 (♀) shows some degree of coincidence also. A corresponding curve of percentage growth is shown for the Cawsand fish (Fig. 17 [A]), the data for which is given in Table 50 (A). Similar periodic fluctuations are seen to obtain, but not altogether independently of sex. It is readily seen by comparison of (A) and (B) sections of Fig. 17

FIG. 18.—Graph of maximum and minimum temperatures at Lympstone (1929).

that the percentage growth is greater in the case of Cawsand males, but smaller in the case of females.

In Fig. 18 is shown a graph of maximum and minimum temperatures of the tank recorded at Lympstone. The temperatures at Lympstone are seen to be consistently much higher from June to September than those obtaining at Cawsand. This fact in part explains the increased appetite and growth of the maximum fish as compared with those at Cawsand and also the slightly higher values of maintenance rations.

4. Conclusions Drawn from the Experiments of 1929 (Lympstone).

(1) Between 0·90 gm. and 0·95 gm. food per day is necessary in order to maintain constant the body-weight of a 50-gm. male fish (L1).

(2) About 1·4 gm. of food per day is necessary in order to maintain constant the body-weight of a 130-gm. male fish. The maintenance requirements of fish 50 gm. to 130 gm. in weight range from 0·9 gm. to 1·4 gm. of food per day.

(3) For female fish 60 gm. to 114 gm. in weight, from 0·8 gm. to 1·5 gm. of food per day is required in order to maintain the weight constant.

(4) The ratio of food required per day for purposes of maintenance/average body-weight of a fish diminishes as the weight of the fish increases, in male and female alike.

(5) Maximum growth for the experiments with male plaice was displayed by L11, which, during a period of 140 days from June 24th, 1929, increased its length from 16·1 cm. to 23·1 cm., its weight from 23·2 gm. to 158·6 gm. It consumed 926 gm. of food, an average of 6·6 gm. per day, and for the whole period, 6·8 gm. of food produced 1·0 gm. of fish. Maximum growth, appetite and efficiency obtained during the same period, September 16th to October 14th.

(6) The female plaice L15 increased its length from 15·1 cm. to 23·0 cm. during 126 days from June 24th, its weight from 31·9 gm. to 141·1 gm. Maximum growth, appetite and efficiency obtained synchronously during late September. The female L16 increased its length from 18·4 cm. to 24·0 cm. during a period of 140 days, its weight from 50·5 gm. to 179·3 gm. From September 16th to October 14th, a period of 28 days, the fish took roughly 250 gm. of food and increased its weight by 38·6 gm., 26 per cent of the weight on September 16th.

(7) Growth of both males and females is abruptly retarded when the maximum temperature of the sea-water approaches 10° C. Growth ceases and loss of weight is incurred when the maximum temperature falls below 10° C.

VI. GENERAL DISCUSSION OF RESULTS.

It was seen that during the preliminary experiments of 1928, the average quantity of food required daily for purposes of maintenance by a mean male fish 44 gm. in weight is approximately 0·6–0·7 gm. But it was suggested that because the decline of a fish which probably failed to obtain

food, the value obtained was undoubtedly a low one, and that in all likelihood 0·7–0·8 gm. per day is a more accurate value. Shown as a ratio of the body-weight the value ranges between 0·017 and 0·019, and was suggested as being approximately constant for this fish at 0·018 (Table 1).

In Table 51 the average daily food ration of the above mean male fish of the 1928 experiments is shown to November 30th. For a fish of average weight 42·1 gm. the daily ration is 0·7 gm., or 0·017 of the average weight. Data are also shown in this table which enable us to compare the above result with the results obtained under more refined conditions during 1929 at Cawsand and at Lympstone. The mean A fish of 1928 best compares with C6a (1929) as regards weight, and close conformity is obtained between the observed values for a daily maintenance ration. It is conclusively shown that for a 42 gm. male fish, i.e. a fish commencing its third season of growth, 0·7 gm. of food or 0·017 of the body-weight, per day is sufficient to maintain the body-weight constant.

For a smaller fish a smaller daily ration is required of course, but the decrease in value of this ration is not directly proportional to the decrease in weight of the fish. Thus, C5a, which is 30 gm. in weight, requires 0·6 gm. of food per day, or 0·019 of the body-weight (Table 51), while the mean C fish of 1929, which is much smaller, weighing 17·6 gm., requires 0·4 gm., or 0·024 of the body-weight of food per day. It is thus rendered clear from comparisons afforded by the results of the Cawsand experiments alone, that the value of the maintenance ratio, i.e. the ratio of food required per day to body-weight, decreases as the weight of the fish increases in the case of males. And this interesting result receives support from the results of the Lympstone experiments with male fish. Thus L1, which weighs 52 gm., requires 0·9 gm. or 0·017 of the body-weight of food per day, while L2, which weighs 130 gm., requires 1·4 gm. or 0·011 of the body-weight of food daily. It is clear that considerable variation in the value of the maintenance ratio must occur during the third growth season of a plaice. If the initial and final weights are, say, 18 gm. and 130 gm., the fraction of the body-weight required as daily ration steadily decreases from 0·024 towards 0·011, the actual weight of food per day increasing from 0·4 gm. to 1·4 gm. In Table 51 several intermediate values are shown, while in Fig. 19 a graph of the variation in maintenance rations with size is shown, as given with the data of the results of Cawsand and Lympstone experiments. As was suggested, the curve is not a straight line one, although the Lympstone data alone gave such a curve. The curve would approximate more closely to a straight line, however, if the temperatures at Lympstone and at Cawsand were identical. The higher temperatures obtaining at Lympstone presumably cause the maintenance requirements to be higher there than at Cawsand.

Comparing the results of the three sets of experiments for the females, it

is seen that, as in the case of males, the fraction of the body-weight of food required daily for purposes of maintenance decreases as the weight of the fish increases. Thus a Cawsand female fish of 22 gm. requires 0·4 gm. of food per day, a similar fish of 42 gm. requires 0·6 gm. per day, while a Lympstone fish of 103 gm. requires 1·3 gm. per day ; 0·019, 0·015 and 0·013 of the body-weight respectively (Table 52). The maintenance requirements of several fish of various sizes between 22 gm. and 114 gm. in weight are also shown in Table 52, and a curve showing the relationship between the daily maintenance ration and size of fish is presented in Fig 19. The curve is open to the same objections as that for the males, but gives

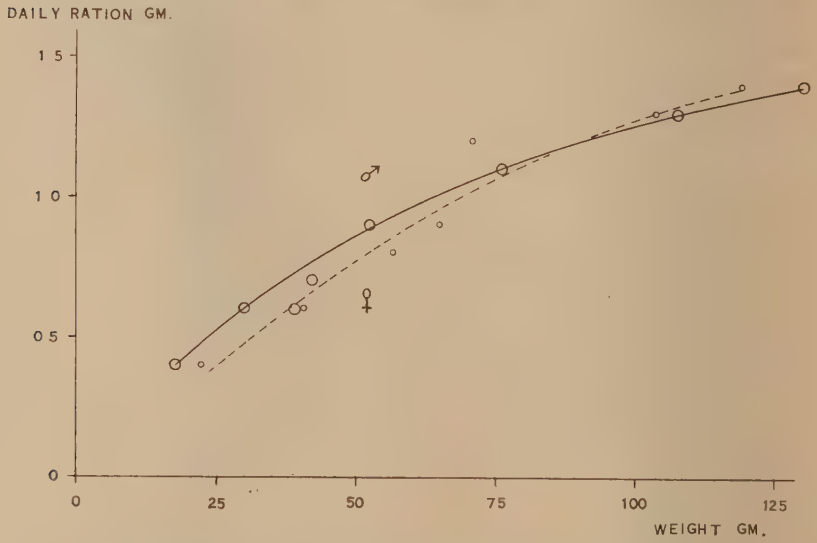

Fig. 19.—Graph showing relationship between daily maintenance ration and size of fish. Based upon Cawsand and Lympstone experiments of 1929.

an approximation of some value. From the evidence obtained, it appears that the maintenance requirements of females are slightly less than those of males, for any fish below 100 gm. in weight.

It must be remembered in spite of the generalisations made above, that the maintenance requirements of any fish appear to vary in an irregular manner from one fortnightly period to another. This obtains for Cawsand and Lympstone fish alike. Examples have been given in the general description of the results and merely is it intended here to emphasise the fact that such variations do occur, although it is not possible to account for them.

One fact stands out clearly when one compares the maximum require-

ments and efficiency of male fish used in the Cawsand experiments of 1928 and 1929, that the maximum requirements of 1928 were much greater, the efficiency much smaller than in the following year (Table 53). The mean male fish of 1928 took 1129 gm. of food, while D3, the most rapidly growing male fish of 1929, took only rather more than half this quantity and yet increased its weight more substantially. The values obtained from the refined experiments of 1929 are undoubtedly more accurate than those from the experiments of the previous year, and the efficiency of the fish is much greater than the experiments of 1928 indicated. Over a period of 175 days, D3 increased its weight by 1·0 gm. for every 6·5 gm. of food it consumed, and took an average of 3·3 gm. of food per day. At Lympstone, where a higher temperature obtained, growth and appetite were greater, while efficiency was slightly lower. The male fish L11 took 953 gm. of food during a period of 154 days, giving an average of 6·2 gm. per day, and increased its weight by 1·0 gm. for every 7·1 gm. of food taken. It can be inferred, therefore, that the efficiency of a fully growing male fish during its third season of growth lies between 6·5 gm. and 7·1 gm. of food per gm. increase in weight. The fish D3 took 9·75 times the average body-weight for the season of food, while L11 took 10·5 times this weight over a slightly shorter period.

Similar results obtain in the case of female fish. The growth rates of males and females in the Cawsand experiments of 1929 are approximately equal as is appetite in the two sexes [cp. D3 (♂), Table 53, and B4 and B1 (♀s), Table 54]. The males show a rather more rapid growth-rate than the females at Lympstone, while at Cawsand in 1928 the reverse was the case. In the females as in the males, growth and appetite were greater at Lympstone than at Cawsand during the season of 1929, and similarly the efficiency was lower. L15 and L16 took 8·1 gm. and 8·5 gm. of food respectively per gm. increase in weight, while B4 and B1 took only 7·2 gm. and 7·6 gm. for a corresponding increase in weight. The efficiency of the male fish is greater than that of the female at Cawsand and Lympstone alike, and the efficiency of any fish is greater than was indicated by the results of the experiments of 1928 (Tables 53 and 54). The Cawsand fish B4 and B1 took 10 times and 9·5 times the average body-weight for the period respectively of food, the Lympstone fish L15 and L16, 10·3 times and 9·4 times this weight of food respectively, which figures correspond closely to those obtained for the males.

It is seen from Tables 53 and 54 that the growth-rate of different individuals varies considerably. It is difficult to understand why such variations occur. The Cawsand fish were trawled, and it might be argued that the treatment they receive during the process has a baneful effect upon certain individuals. But the Lympstone fish were seined and yet the variations are equally well marked here also. It may be that such

variations in the growth-rate obtain under truly natural conditions. The fish D3 suffers a retardation of growth during mid-August, and also shows a fall in appetite for food (Fig. 5). There is also a fall in temperature during mid-August (Fig. 11, 1929). The retardation is temporary and is followed by an acceleration at a time when the temperature is rising. The fish D2 shows an almost complete inhibition of growth in weight during mid-August, which is followed by a very great acceleration, while the temperature of the pond is still rising. D4 shows a growth retardation during August and no acceleration follows it. It is not justifiable to assume that because two events occur simultaneously one necessarily acts as a cause to the other, and it may not be the case that growth retardation or inhibition was caused by a fall in temperature of the pond in the above instances, but it is reasonable to assume that the same causes influence different individuals to different degrees and that in the above instances variations in the growth-rate of fish not apparently injured in any way are the outcome of natural causes, rather than the results of accident during the process of trawling or seining. It is thus rendered necessary to ascertain the variations in the growth-rate occurring under truly natural conditions, which task lies outside the scope of this work.

The question of the relationship existing between the quantity of food taken and growth in weight remains unanswered. Over a period of 150–175 days the efficiency of fish showing a markedly rapid growth-rate varies but slightly. But that of different individuals varies very considerably. Even in the same fish, good, bad or indifferent, it varies considerably from time to time. In this connexion it is important to note the measure of efficiency for each period for D3 (Table 20), B1 (Table 23), B4 (Table 24), L11 (Table 42), L15 (Table 46), and L16 (Table 47), which may all be taken as " normal " fish showing typical growth curves of length and weight. The variations in weight increments added from period to period in these " normal " fish are well beyond reach of the error of the experiments. It is seen in the results of the Cawsand experiments, however, that the acceleration curves for food and weight increments show an approximate degree of correlation (Figs. 5 and 7) which suggests that the efficiency of these fish varies fairly closely about a mean. Thus, for B1, a female fish, the efficiency from May 22nd to October 1st, 1929, varies between 5·2 gm. and 7·1 gm. of food per gm. increase in weight of the fish. That of the male D3 to October 15th varies between 4·8 gm. and 9·8 gm. of food per gm. of fish. Such correlation is not found in the Lympstone results (Figs 13 and 15), where appetite is seen to be maintained irrespective of growth acceleration or retardation. Thus, the efficiency of L11 to October 14th varies between 2·3 gm. and 17·8 gm. of food per gm. of fish, while for the same period and the same measure of efficiency being understood, the figures for L15 range from 4·9 to 15·1, those for

L16 from 5·7 to 9·4 (the growth-rate here being uniformly rapid). Thus it is not possible to postulate even an approximately constant index of efficiency for any short period during the growth season.

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TABLE 1.

Data Obtained from the Preliminary Experiment on Maintenance Requirements of Male Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. A1. Lth.	A1. Wt.	A2. Lth.	A2. Wt.	A3 Lth.	A3 Wt.	A4. Lth.	A4. Wt	A5. Lth,	A5. Wt.	A6. Lth.	A6. Wt.	Mean A fish. Lth.	Mean A fish. Wt.	No. of days since previous weighing.	Total average food per fish per period (gm.).	Food per fish per day during period (gm.).	Mean fish (neglecting No. 1.). Lth.	Mean fish (neglecting No. 1.). Wt.	Total average quantity of food per fish (of 5). gm.	Food per Fish per day (neglecting No. 1). gm.	Ratio food per day body wt.
July 16	16·8	44·0	16·2	47·0	16·5	45·0	16·1	43·0	14·4	28·0	16·4	45·0	16·1	42·0	–	—	—	15·9	41·6	—	—	—
„ 30	16·7	42·0	16·2	48·0	16·7	48·0	16·4	43·0	14·4	27·0	16·6	43·0	16·2	41·8	14	7·0	0·5	16·1	41·8	8·4	0·6	0·014
Aug. 14	16·9	38·0	16·4	45·0	17·0	50·0	16·4	48·0	14·4	25·0	16·6	40·0	16·3	41·0	15	6·5	0·4	16·2	41·6	7·8	0·5	0·012
„ 27	16·9	37·0	16·5	41·0	17·0	50·0	16·7	52·0	14·4	23·0	16·6	39·0	16·3	40·3	13	9·0	0·7	16·2	41·0	10·8	0·8	0·019
Sept. 10	16·7	35·0	16·4	42·0	17·0	51·0	16·9	54·0	14·4	22·0	16·6	39·0	16·3	40·5	14	9·0	0·6	16·3	41·6	10·8	0·8	0·019
„ 26	16·7	34·0	16·5	41·0	16·9	55·0	16·9	50·0	14·4	21·0	16·6	40·0	16·3	40·2	16	9·0	0·6	16·3	41·4	10·8	0·7	0·017
Oct. 12	16·8	31·0	16·6	44·0	16·9	54·0	16·9	48·0	14·5	21·0	16·7	42·0	16·4	40·0	16	9·7	0·6	16·3	41·8	11·7	0·7	0·017
Nov. 1	16·8	29·0	16·8	48·0	17·1	54·0	16·9	48·0	14·4	22·0	17·0	43·0	16·5	40·7	20	12·7	0·6	16·4	43·0	15·3	0·8	0·019
„ 16	16·8	26·0	16·8	44·0	17·2	53·0	16·9	47·0	14·5	18·0	16·9	40·0	16·5	38·0	15	9·7	0·6	16·5	40·4	11·7	0·8	0·019
„ 30	Dead		16·9	48·0	17·2	55·0	17·0	48·0	14·5	20·0	16·8	42·0	16·5	42·6	14	9·7	0·7	16·5	42·6	10·2	0·7	0·017
Dec. 20 1929			17·1	50·0	17·3	57·0	17·2	52·0	14·6	22·0	16·9	45·0	16·6	45·2	20	11·2	0·6	16·6	45·2	11·2	0·6	0·014
Jan. 8			17·3	52·0	17·4	59·0	17·4	52·0	14·6	25·0	17·0	46·0	16·7	46·8	19	13·8	0·7	16·7	46·8	13·8	0·7	0·015

TABLE 2.

Data Obtained from the Preliminary Experiment on the Maintenance Requirements of Female Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. B1. Lth.	B1. Wt.	B2. Lth.	B2. Wt.	B3. Lth.	B3. Wt.	B4. Lth.	B4. Wt.	B5. Lth.	B5. Wt.	B6. Lth.	B6. Wt.	Mean fish. Lth.	Mean fish. Wt.	Days in each period.	Average quantity of food per fish per period (gm.).	Food per fish per day (gm.).	Ratio food per day / average body-wt. (for period)
July 16	15·7	35·0	12·3	21·0	13·4	24·5	12·8	21·0	12·6	18·5	13·7	26·0	13·4	24·3	—	—	—	—
„ 30	15·7	35·0	12·8	24·0	13·8	29·0	12·8	20·0	12·7	15·5	14·0	27·5	13·6	25·2	14	7·0	0·5	0·020
Aug. 14	15·7	33·0	13·2	27·0	14·0	30·0	12·8	18·0	12·6	15·0	14·3	29·0 [? 29·0]	13·8	25·3 ?	15	6·5	0·4	0·016
„ 27	15·7	31·0	13·8	33·0	14·5	33·0	12·9	17·0	12·7	15·0	14·6	39·0	14·0	26·7	13	9·0	0·7	0·027
Sept. 10	15·8	29·0	14·4	36·0	14·6	34·0	12·7	16·0	12·6	15·0	14·8	32·0	14·1	27·0	14	9·0	0·6	0·022
„ 26	15·7	29·0	14·7	36·0	14·9	37·0	12·8	17·0	12·7	14·0	15·3	35·0	14·3	28·0	16	9·0	0·6	0·022
Oct. 12	15·6	29·0	15·0	35·0	14·9	39·0	12·9	15·0	12·7	14·0	15·5	37·0	14·4	28·3	16	9·7	0·6	0·021
Nov. 1	15·7	28·0	15·0	38·0	15·4	45·0	12·7	15·0	Dead		15·6	40·0	14·9	33·2	20	12·7	0·6	0·020
„ 16	Dead		15·1	39·0	15·4	34·0	12·9	17·0			15·9	40·0	14·8	32·5	15	9·7	0·6	0·018
„ 30			15·4	38·0	15·8	38·0	13·2	19·0			15·6	45·0	15·0	35·0	14	9·7	0·7	0·021
Dec. 20			15·6	41·0	16·1	41·0	13·4	23·0			15·8	47·0	15·2	38·0	20	11·2	0·6	0·016
1929 Jan. 8			15·8	44·0	16·4	44·0	13·8	25·0			16·3	51·0	15·6	41·0	19	12·7	0·7	0·018

TABLE 3.

Data Obtained from an Experiment on Maximum Feeding and Growth in Male Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. C1. Lth.	C1. Wt.	C2. Lth.	C2. Wt.	C3. Lth.	C3. Wt.	C4. Lth.	C4. Wt.	C5. Lth.	C5. Wt.	C6. Lth.	C6. Wt.	Mean C fish. Lth.	Mean C fish. Wt.	Mean growth per period Lth.	Mean growth per period Wt.	No. of days since previous weighing.	Average weight of food per fish per period (gm.).	Maintenance allowance per period (= Bdy.-Wt. × M × days) (gm.).	Food per period available for growth (gm.).	Food available for growth (cumulative) (to 1 gm.).	Cumulative growth. Lth.	Cumulative growth. Wt.
July 16	16·8	46·0	15·6	36·0	16·2	37·0	15·4	30·0	16·4	42·0	15·9	42·0	16·0	38·8	—		—	—	—	—	—	—	
„ 30	16·9	53·0	16·5	39·0	16·9	42·0	15·8	38·0	17·2	50·0	16·2	42·0	16·6	44·0	0·6	5·2	14	71·7	7·6	64·1	64	0·6	5·2
Aug. 14	18·1	69·0	16·7	42·0	17·1	53·0	16·2	43·0	18·2	62·0	17·2	45·0	17·2	52·3	0·6	8·3	15	65·0	7·9	57·1	121	1·2	13·5
„ 27	19·6	96·0	17·2	53·0	17·8	58·0	16·5	47·0	19·0	73·0	17·9	52·0	18·0	63·2	0·8	10·9	13	90·0	12·9	77·1	198	2·0	24·4
Sept. 10	21·0	115·0	18·0	60·0	18·6	70·0	16·8	48·0	19·7	80·0	18 1	55·0	18·7	71·3	0·7	8·1	14	90·0	16·8	73·2	271	2·7	32·5
„ 26	22·4	137·0	18·8	66·0	19·8	84·0	17·2	55·0	20·3	86·0	18·5	62·0	19·5	81·7	0·8	10·4	16	90·0	19·4	70·6	342	3·5	42·9
Oct. 12	—		—		20·1	87·0	—		20·9	95·0	18·5	59·0	19·5	80·2	—	—	16	97·5	22·2	75·3	—	—	—
Nov. 1					21·2	101·0			21·1	100·0	18·8	59·0	20·4	86·7	0·9	6·5	20	127·5	30·5	97·0	97	0·9	6·5
„ 16					21·7	116·0			21·4	101·0	19·4	68·0	20·8	95·0	0·4	8·3	15	97·5	24·7	72·4	169	1·3	14·8
„ 30					22·6	128·0			21·8	104·0	19·8	76·0	21·4	102·7	0·6	7·7	1 4	97·5	22·6	74·9	244	1·9	22·5
Dec. 20 1929					23·5	151·0			22·1	115·0	20·6	88·0	22·1	118·0	0·7	15·3	20	133·5	28·8	104·7	349	2·6	37·8
Jan. 8					24·1	158·0			22·6	117·0	21·0	95·0	22·6	123·3	0·5	5·3	19	169·0	33·6	135·4	484	3·1	43·1
															3·5	42·9						3·5	42·9
													Totals		3·1	43·1	176	1129·2	227·4			3·1	43·1

TABLE 4.

Data Obtained from an Experiment on Maximum Feeding and Growth in Female Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. D1. Lth.	D1. Wt.	D2. Lth.	D2. Wt.	D3. Lth.	D3. Wt.	D4. Lth.	D4. Wt.	D5. Lth.	D5. Wt.	D6. Ltd.	D6. Wt.	Mean D fish. Lth.	Mean D fish. Wt.	Mean growth per period. Lth.	Mean growth per period. Wt.	No. of days since previous weighing.	Average weight of food per fish per period (gm.).	Average weight of food per fish per day (gm.).
July 16	16·4	48·0	15·2	38·0	16·6	43·0	15·6	44·0	16·7	50·0	16·1	37·0	16·1	43·3	—	—	—	—	
,, 30	17·5	62·5	16·4	50·0	17·4	57·0	16·5	54·0	17·7	68·0	16·7	44·5	17·0	56·0	0·9	12·7	14	71·7	5·1
Aug. 14	18·8	80·0	17·3	57·0	18·8	76·0	17·7	66·0	18·9	81·0	17·3	50·0	18·1	68·3	1·1	12·3	15	65·0	4·4
,, 27	19·8	95·0	18·8	64·0	19·6	88·0	18·5	79·0	19·9	96·0	18·0	54·0	19·1	79·3	1·0	11·0	13	90·0	6·9
Sept. 10	20·6	100·0	19·1	79·0	20·5	105·0	19·7	95·0	21·0	116·0	18·7	63·0	19·9	93·0	0·8	13·7	14	90·0	6·4
,, 26	21·0	113·0	19·4	88·0	21·0	118·0	20·5	112·0	22·4	137·0	19·4	77·0	20·6	107·5	0·7	14·5	16	90·0	5·6
Oct. 12	21·5	127·0	20·5	103·0	21·7	131·0	21·3	111·0	23·2	155·0	20·1	83·0	21·4	118·3	0·8	10·8	16	97·5	6·1
Nov. 1	22·7	150·0	21·1	101·0	22·8	150·0	21·9	122·0	23·8	165·0	20·8	91·0	22·2	129·8	0·8	11·5	20	127·5	6·4
,, 16	22·9	157·0	21·2	100·0	23·1	149·0	22·1	124·0	24·3	175·0	21·0	102·0	22·4	134·5	0·2	4·7	15	97·5	6·5
,, 30	23·3	164·0	21·5	104·0	23·1	150·0	22·1	123·0	24·7	197·0	21·2	103·0	22·6	140·2	0·2	5·7	14	97·5	7·0
Dec. 20	23·8	176·0	22·3	114·0	23·5	155·0	22·6	128·0	25·2	205·0	21·2	104·0	23·1	147·0	0·5	6·8	20	133·5	6·7
1929 Jan. 8	24·1	186·0	22·6	121·0	23·4	157·0	22·9	128·0	25·6	203·0	21·3	112·0	23·3	151·2	0·2	4·2	19	169·0	8·9
													Totals		7·2	107·9	176	1129·2	

TABLE 5.

Data Obtained from an Experiment on Intermediate Feeding and Growth in Male Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. E1. Lth.	E1. Wt.	E2. Lth.	E2. Wt.	E3. Lth.	E3. Wt.	E4. Lth.	E4. Wt.	E5. Lth.	E5. Wt.	E6. Lth.	E6. Wt.	Mean E fish. Lth.	Mean E fish. Wt.	Mean growth per period. Lth.	Mean growth per period. Wt.	No. of days since previous weighing.	Average weight of food per fish per period (gm.).	Average weight of food per fish per day (gm.).
July 16	17·2	53·0	14·0	25·0	17·0	54·0	16·6	45·0	15·8	41·0	15·7	37·0	16·0	42·5	—		—	—	–
„ 30	17·9	71·0	13·9	24·0	18·5	72·0	17·0	49·0	16·9	55·0	15·9	37·0	16·7	51·3	0·7	8·8	14	34·9	2·5
Aug. 14	19·0	81·0	14·0	23·0	19·7	91·0	17·0	48·0	17·9	65·0	15·8	45·0	17·2	58·8	0·5	7·5	15	32·5	2·2
„ 27	19·7	89·0	14·0	20·0	20·4	98·0	17·1	48·0	18·2	68·0	16·2	42·0	17·6	60·8	0·4	2·0	13	30·0	2·3
Sept. 10	20·4	100·0	13·8	22·0	21·0	99·0	17·1	48·0	18·7	73·0	16·7	49·0	17·9	65·2	0·3	4·4	14	30·0	2·1
„ 26	20·9	112·0	14·0	20·0	21·2	104·0	17·3	56·0	18·9	75·0	17·0	48·0	18·2	69·1	0·3	3·9	16	30·0	1·9
Oct. 12	21·3	120·0	13·9	19·0	21·5	110·0	18·1	66·0	19·0	74·0	17·1	48·0	18·5	72·8	0·3	3·7	16	32·5	2·0
Nov. 1	21·8	123·0	13·8	20·0	22·1	120·0	19·0	74·0	19·2	80·0	17·1	47·0	18·8	77·3	0·3	4·5	20	42·5	2·1
„ 16	22·0	130·0	13·9	15·0	22·3	120·0	19·6	86·0	19·6	88·0	17·1	48·0	19·1	81·2	0·3	3·9	15	32·5	2·2
„ 30	22·5	134·0	14·3	21·0	22·4	133·0	19·9	86·0	—	—	17·1	45·0	19·2	83·8	0·1	2·6	14	32·5	2·3
Dec. 20	22·5	142·0	14·5	27·0	22·8	129·0	20·3	95·0			17·1	47·0	19·4	88·0	0·2	4·2	20	45·0	2·2
1929 Jan. 8	22·9	149·0	15·0	32·0	22·8	134·0	21·0	106·0			17·1	47·0	19·8	93·6	0·4	5·6	19	51·0	2·7
														Totals	3·8	51·1	176	393·4	

TABLE 6.

Data Obtained from an Experiment on Intermediate Feeding and Growth in Female Plaice.

Date. (1928)	Fish Nos., Lengths and Weights. F1. Lth.	F1. Wt.	F2. Lth.	F2. Wt.	F3. Lth.	F3. Wt.	F4. Lth.	F4. Wt.	F5. Lth.	F5. Wt.	F6. Ltd.	F6. Wt.	Mean F fish. Lth.	Mean F fish. Wt.	Mean growth per period. Lth.	Mean growth per period. Wt.	No. of days since previous weighing.	Average weight of food per fish per period (gm.).	Average weight of food per fish per day (gm.).
July 16	17·1	50·0	16·0	39·0	16·7	46·0	16·9	47·0	15·8	39·0	15·1	30·0	16·3	41·8	—			—	—
„ 30	18·0	61·0	17·0	52·0	17·1	54·0	17·4	54·0	16·0	37·0	15·4	33·0	16·8	48·5	0·5	6·7	14	35·8	2·6
Aug. 14	18·2	64·0	17·5	58·0	18·3	62·0	18·5	62·0	16 1	35·0	15·8	36·0	17·4	52·8	0·6	4·3	15	32·5	2·2
„ 27	18·3	69·0	17·9	66·0	18·4	67·0	18·4	61·0	16·4	43·0	15·9	37·0	17·5	57·2	0·1	4·4	13	30·0	2·3
Sept. 10	19·0	71·0	18·5	73·0	19·0	69·0	18·5	62·0	17·0	52·0	16·1	39·0	18·0	61·0	0·5	3·8	14	30·0	2·1
„ 26	19·3	84·0	19·1	75·0	19·0	74·0	18·2	61·0	17·7	63·0	16·3	42·0	18·3	66·5	0·3	5·5	16	30·0	1·9
Oct. 12	19·9	88·0	19·2	75·0	19·1	74·0	18·6	71·0	18·3	58·0	16·4	46·0	18·6	68·7	0·3	2·2	16	32·5	2·0
Nov. 1	20·6	100·0	19·6	78·0	19·5	75·0	19·2	81·0	18·3	59·0	16·7	46·0	19·0	73·2	0·4	4·5	20	42·5	2·1
„ 16	20·9	106·0	19·9	82·0	19·6	75·0	19·7	86·0	18·3	59·0	16·8	47·0	19·2	75·8	0·2	2·6	15	32·5	2·2
„ 30	21·3	115·0	19·9	83·0	20·1	89·0	20·0	93·0	18·5	60·0	16·9	50·0	19·4	81·7	0·2	5·9	14	32·5	2·3
Dec. 20	21·8	118·0	20·3	91·0	20·4	93·0	20·6	104·0	18·5	61·0	17·0	51·0	19·8	86·3	0·4	4·6	20	37·5	1·9
1929 Jan. 8	22·3	126·0	20·7	95·0	21·1	103·0	21·5	110·0	19·1	74·0	17·4	56·0	20·3	94·0	0·5	7·7	19	38·5	2·0
														Totals	4·0	52·2	176	374·3	

TABLE 7.

Data Obtained from the Preliminary Experiments.

Showing initial and final values for length and weight of the mean fish in each group and also the corresponding increases. Total quantities of food supplied are shown, as is a measure of the efficiency of each mean fish, in terms of gm. of food effecting 1·00 gm. increase in fish weight.

	Males.			Females.		
	Minimum Fed.	Maximum Fed.	Intermediate Fed.	Minimum Fed.	Maximum Fed.	Intermediate Fed.
Initial length (cm.)	16·1	16·0	16·0	13·4	16·1	16·3
Final length	16·7	22·6	19·8	15·6	23·3	20·3
Growth in length	0·6	6·6	3·8	2·2	7·2	4·0
Initial weight (gm.)	42·0	38·8	42·5	24·3	43·3	41·8
Final weight	46·8	123·3	93·6	41·0	151·2	94·0
Growth in weight	4·8	84·5	51·1	16·7	107·9	52·2
Total food consumed (gm.)	107	1129	393	106	1129	374
Gm. of food per 1·0 gm. increase in fish weight	[22·3]	13·4	7·7	[6·37]	10·5	7·2

TABLE 8.

Data Obtained from Experiments on the Maintenance Requirements of Male and Female Plaice.

The experiments extended over a period of 44 days, from May 22nd to July 5th, 1929.

Fish No.	Sex.	Initial size.		Final size.		Growth for period.		Total food per the period (44 days).	Average weight of food per day. gm.	Ratio food per day. / Average body-wt. (for period)
		Lth.	Wt.	Lth.	Wt.	Lth.	Wt.			
C1	♂	12·8	17·5	13·2	21·5	0·4	4·0	18·6	0·42	0·022
C2	♂	12·6	16·5	13·3	20·5	0·7	4·0	18·3	0·42	0·023
C3	♂	12·7	18·0	12·9	18·0	0·2	nil	18·6	0·42	0·023
C4	♂	12·8	18·5	12·9	15·5	0·1	− 3·0	19·0	0·43	0·025
C5	♂	12·3	15·0	12·8	18·0	0·5	3·0	18·3	0·42	0·025
C6	♂	12·8	14·5	12·9	17·0	0·1	2·5	18·3	0·42	0·027
Mean fish	♂	12·7	16·7	13·0	18·4	0·3	1·7[5]	18·5	0·42	0·024
A1	♀	13·4	20·5	13·6	22·0	0·2	1·5	18·3	0·42	0·020
A2	♀	13·5	22·5	13·8	24·0	0·3	1·5	18·3	0·42	0·018
A3	♀	13·5	21·0	13·8	22·0	0·3	1·0	18·6	0·42	0·020
A4	♀	13·7	21·0	13·9	22·0	0·2	1·0	18·6	0·42	0·020
A5	♀	13·8	23·5	14·3	23·5	0·5	nil	18·6	0·42	0·018
A6	♀	13·8	24·0	13·9	22·0	0·1	− 2·0	18·9	0·43	0·019
Mean fish	♀	13·6	22·1	13·9	22·6	0·3	0·5	18·5[5]	0·42	0·019

TABLES 9–11

Data Obtained from the Experiment on the Maintenance Requirements of Male Fish

Table 9: C5a.

Date (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Average wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
July 5	14·4	30·5	—		—	—	—	—	—	—		—
„ 19	14·4	26·0	nil	− 4·5	4·75	14	0·34	4·75	14	0·34	nil.	− 4·5
Aug. 2	14·5	25·0	0·1	− 1·0	5·4	14	0·39	10·15	28	0·36	0·1	− 5·5
„ 16	14·5	23·5	nil	− 1·5	8·25	14	0·59	18·40	42	0·44	0·1	− 7·0
„ 30	14·5	25·5	nil	2·0	9·00	14	0·64	27·40	56	0·49	0·1	− 5·0
Sept. 16	14·5	27·0	nil	1·5	11·25	17	0·66	38·65	73	0·53	0·1	− 3·5
Oct. 1	14·7	28·0	0·2	1·0	12·0	15	0·80	50·65	88	0·58	0·3	− 2·5
„ 15	14·7	29·0	nil	1·0	10·0	14	0·71	60·65	102	0·59	0·3	− 1·5
„ 29	14·8	30·0	0·1	1·0	8·0	14	0·57	68·65	116	0·59	0·4	− 0·5
Nov. 13	14·8	29·5	nil	− 0·5	7·5	15	0·50	76·15	131	0·58	0·4	− 1·0
„ 28	14·8	29·5	nil	nil	5·5	15	0·4	81·65	146	0·56	0·4	− 1·0

Table 10: C4a.

July 12	15·6	36·0	—		—	—	—	—	—	—	—	
„ 19	15·6	34·5	nil	− 1·5	1·7	7	0·24	1·7	7	0·24	nil	− 1·5
Aug. 2	15·5	33·0	− 0·1	− 1·5	3·4	14	0·24	5·1	21	0·24	− 0·1	− 3·0
„ 16	15·6	33·5	0·1	0·5	6·0	14	0·43	11·1	35	0·32	nil	− 2·5
„ 30	15·7	34·0	0·1	0·5	12·0	14	0·86	23·1	49	0·47	0·1	− 2·0
Sept. 16	15·7	35·0	nil	1·0	12·5	17	0·74	35·6	66	0·54	0·1	− 1·0
Oct. 1	15·8	38·0	0·1	3·0	12·5	15	0·83	48·1	81	0·60	0·2	2·0
„ 15	16·0	39·0	0·2	1·0	10·0	14	0·71	58·1	95	0·61	0·4	3·0
„ 29	16·2	40·0	0·2	1·0	8·5	14	0·61	66·6	109	0·61	0·6	4·0
Nov. 13	16·2	42·0	nil	2·0	8·2	15	0·55	74·8	124	0·60	0·6	6·0

Table 11: C6a.

July 12	16·3	41·5	—		—	—	—	—	—	—	—	
„ 19	16·4	40·0	0·1	− 1·5	1·8	7	0·26	1·8	7	0·26	0·1	− 1·5
Aug. 2	16·4	38·0	nil	− 2·0	8·0	14	0·57	9·8	21	0·47	0·1	− 3·5
„ 16	16·5	40·0	0·1	2·0	12·3	14	0·88	22·1	35	0·63	0·2	− 1·5
„ 30	16·5	39·5	nil	− 0·5	13·0	14	0·93	35·1	49	0·72	0·2	− 2·0
Sept. 16	16·6	40·5	0·1	1·0	15·0	17	0·88	50·1	66	0·76	0·3	− 1·0
Oct. 1	16·8	41·0	0·2	0·5	12·5	15	0·83	62·6	81	0·77	0·5	− 0·5
„ 15	16·8	43·0	nil	2·0	12·0	14	0·86	74·6	95	0·79	0·5	1·5
„ 29	16·9	44·0	0·1	1·0	8·0	14	0·57	82·6	109	0·76	0·6	2·5
Nov. 13	17·0	45·0	0·1	1·0	8·0	15	0·53	90·6	124	0·73	0·7	3·5
„ 28	17·0	44·5	nil	− 0·5	5·5	15	0·37	96·1	139	0·69	0·7	3·0

TABLE 12.

Data Obtained from the Experiments on the Maintenance Requirements of the Male Plaice, C5a, C4a, and C6a.

Date. (1929)	No. of days in period.	30·5 C5a Increase in wt. per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).‡	36·0 C4a Increase in wt. per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).	41·5 C6a Increase in wt. per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).
July 5*	—	—	—	—	—	—	—	—	—	—
,, 19	14†	− 4·5	0·012	0·012	− 1·5	0·007	0·007	− 1·5	0·006	0·006
Aug. 2	14	− 1·0	0·015	0·013	− 1·5	0·007	0·007	− 2·0	0·015	0·012
,, 16	14	− 1·5	0·024	0·016	0·5	0·013	0·009	2·0	0·023	0·015
,, 30	14	2·0	0·026	0·017	0·5	0·026	0·013	− 0·5	0·023	0·018
Sept. 16	17	1·5	0·025	0·018	1·0	0·021	0·015	1·0	0·022	0·019
Oct. 1	15	1·0	0·029	0·020	3·0	0·023	0·016	0·5	0·020	0·019
,, 15	14	1·0	0·025	0·020	1·0	0·018	0·016	2·0	0·020	0·019
,, 29	14	1·0	0·019	0·020	1·0	0·015	0·016	1·0	0·013	0·018
Nov. 13	15	− 0·5	0·017	0·019	2·0	0·013	0·015	1·0	0·012	0·017
,, 28	15	nil	0·014	0·019	—	—	—	− 0·5	0·008	0·016

* July 12th in cases of C4a and C6a.
† Seven days in cases of C4a and C6a.
‡ By mid body-weight is meant the mid value between the initial and final body-weights.

TABLES 13–15.

Data Obtained from the Experiment on the Maintenance Requirements of Female Fish.

Table 13: A4a.

Date (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Total wt. of food to date. (gm.)	No. of days. since commencement of expt.	Average. wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
July 5	15·8	39·0	—		—	—	—	—	—	—	—	
,, 19	15·8	32·0	nil	− 7·0	5·7	14	0·40	5·7	14	0·40	nil	− 7·0
Aug. 2	15·9	33·0	0·1	1·0	9·8	14	0·70	15·5	28	0·55	0·1	− 6·0
,, 16	16·0	34·5	0·1	1·5	10·0	14	0·71	25·5	42	0·61	0·2	− 4·5
,, 30	16·1	35·0	0·1	0·5	10·5	14	0·75	36·0	56	0·64	0·3	− 4·0
Sept. 16	16·2	37·0	0·1	2·0	12·5	17	0·74	48·5	73	0·66	0·4	− 2·0
Oct. 1	16·3	38·0	0·1	1·0	12·0	15	0·80	60·5	88	0·69	0·5	− 1·0
,, 15	16·5	40·0	0·2	2·0	10·5	14	0·75	71·0	102	0·70	0·7	1·0
,, 29	16·7	40·5	0·2	0·5	8·0	14	0·57	79·0	116	0·68	0·9	1·5
Nov. 13	16·8	42·0	0·1	1·5	8·5	15	0·57	87·5	131	0·67	1·0	3·0
,, 27	16·8	42·0	nil	nil	5·5	14	0·39	93·0	145	0·64	1·0	3·0

Table 14 : A6a.

Date (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Average wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
July 5	15·7	38·0	—		—	—	—	—	—	—	—	
„ 19	15·8	34·5	0·1	− 3·5	5·6[5]	14	0·40	5·6	14	0·40	0·1	− 3·5
Aug. 2	15·8	36·5	nil	2·0	8·7	14	0·62	14·3	28	0·51	0·1	− 1·5
„ 16	15·9	37·0	0·1	0·5	9·5[5]	14	0·68	23·9	42	0·57	0·2	− 1·0
„ 30	16·0	38·0	0·1	1·0	10·5	14	0·75	34·4	56	0·61	0·3	nil
Sept. 16	16·2	37·0	0·2	− 1·0	12·5	17	0·73	46·9	73	0·64	0·5	− 1·0
Oct. 1	16·4	41·0	0·2	4·0	11·0	15	0·73	57·9	88	0·66	0·7	3·0
„ 15	16·6	42·0	0·2	1·0	10·0	14	0·71	67·9	102	0·67	0·9	4·0
„ 29	16·7	43·5	0·1	1·5	8·0	14	0·57	75·9	116	0·65	1·0	5·5
Nov. 13	16·9	45·0	0·2	1·5	8·0	15	0·53	83·9	131	0·64	1·2	7·0
„ 27	16·9	46·0	nil	1·0	5·5	14	0·39	89·4	145	0·62	1·2	8·0

Table 15 : A3a.

July 12	17·6	55·0	—		—	—	—	—	—	—	—	
„ 19	17·6	54·5	nil	− 0·5	3·0	7	0·43	3·0	7	0·43	nil	− 0·5
Aug. 2	17·8	54·0	0·2	− 0·5	9·8	14	0·70	12·8	21	0·61	0·2	− 1·0
„ 16	18·0	54·5	0·2	0·5	13·5	14	0·96	26·3	35	0·75	0·4	− 0·5
„ 30	17·9	55·0	− 0·1	0·5	12·0	14	0·86	38·3	49	0·78	0·3	nil
Sept. 16	17·8	55·0	− 0·1	nil	13·5	17	0·80	51·8	66	0·78	0·2	nil
Oct. 1	17·8	57·5	nil	2·5	12·0	15	0·80	63·8	81	0·79	0·2	2·5
„ 15	17·9	60·0	0·1	2·5	19·5	14	1·39	83·3	95	0·88	0·3	5·0
„ 29	17·9	58·5	nil	− 1·5	14·5	14	1·04	97·8	109	0·90	0·3	3·5
Nov. 13	17·9	60·0	nil	1·5	10·0	15	0·67	107·8	124	0·87	0·3	5·0
„ 27	17·9	58·0	nil	− 2·0	5·5	14	0·39	113·3	138	0·82	0·3	3·0

TABLE 16.

Data Obtained from the Experiments on the Maintenance Requirements of the Female Plaice A4a, A6a, and A3a.

Date. (1929)	No. of days in period.	39·0 Increase in wt. per period. (gm.)	**A4a** Food per day/ /average body-wt. (for each period).	42·0 Food per day/ /mid body-wt. (to date).	38·0 Increase in wt. per period. (gm.)	**A6a** Food per day/ /average body-wt. (for each period).	46·0 Food per day/ /mid body-wt. (to date).	55·0 Increase in wt. per period. (gm.)	**A3a** Food per day/ /average Lody-wt. for each period).	58·0 Food per day/ /mid body-wt. (to date).
July 5*	—	—	—	—	—	—	—	—	—	—
„ 19	14†	− 7·0	0·011	0·011	− 3·5	0·011	0·011	− 0·5	0·008	0·008
Aug. 2	14	1·0	0·022	0·015	2·0	0·017	0·014	− 0·5	0·013	0·011
„ 16	14	1·5	0·021	0·017	0·5	0·019	0·015	0·5	0·018	0·014
„ 30	14	0·5	0·022	0·017	1·0	0·020	0·016	0·5	0·016	0·014
Sept. 16	17	2·0	0·021	0·017	− 1·0	0·019	0·017	nil	0·015	0·014
Oct. 1	15	1·0	0·021	0·018	4·0	0·019	0·017	2·5	0·014	0·014
„ 15	14	2·0	0·019	0·018	1·0	0·017	0·017	2·5	0·024	0·015
„ 29	14	0·5	0·014	0·017	1·5	0·013	0·016	− 1·5	0·018	0·016
Nov. 13	15	1·5	0·014	0·017	1·5	0·012	0·015	1·5	0·011	0·015
„ 27	14	nil	0·009	0·016	1·0	0·009	0·015	− 2·0	0·007	0·015

* July 12th in case of A3. † Seven days in case of A3.

TABLE 17.

Summarised Data from the Experiments on Maintenance in the Male Plaice, C5a, C4a, and C6a, and in the Female Plaice, A4a, A6a, and A3a.

Fish No.	Sex.	Initial size. Lth.	Initial size. Wt.	Final size. Lth.	Final size. Wt.	Growth. Lth.	Growth. Wt.	Period. (1929).	Days in period.	Average weight of food per day for period. (gm.).	Ratio Food per day / average body-weight for period.
C5a	♂	14·4	30·5	14·8	29·5	0·4	−1·0	July 5th to Nov. 28th	146	0·56	0·019
C4a	♂	15·6	36·0	16·2	42·0	0·6	6·0	July 12th to Nov. 13th	124	0·60	0·015
C6a	♂	16·3	41·5	17·0	44·5	0·7	3·0	July 12th to Nov. 28th	139	0·69	0·016
A4a	♀	15·8	39·0	16·8	42·0	1·0	3·0	July 5th to Nov. 27th	145	0·64	0·016
A6a	♀	15·7	38·0	16·9	46·0	1·2	8·0	July 5th to Nov. 27th	145	0·62	0·015
A3a	♀	17·6	55·0	17·9	58·0	0·3	3·0	July 12th to Nov. 27th	138	0·82	0·015

TABLE 18.

Showing how the Maintenance Requirements of Male and Female Plaice vary with the size of Fish and how these Requirements Diminish During late November.

1929.		October 1st–November 13th (43 days).									November 13th–27th (14 days)								
		Initial size.		Final size.		Growth.		Total weight of food per period. (gm.)	Average weight of food per day for period. (gm.)	Ratio food per day / average body-wt. (for period)	Initial size.		Final size.		Growth.		Total weight of food per period. (gm.)	Average weight of food per day for period. (gm.)	Ratio food per day / average body-wt. (for period)
Fish No.	Sex.	Lth.	Wt.	Lth.	Wt.	Lth.	Wt.				Lth.	Wt.	Lth.	Wt.	Lth.	Wt.			
C2a	♂	19·6	65·0	19·5	64·0	− 0·1	− 1·0	30·0	0·70	0·011	19·5	64·0	19·4	65·0	− 0·1	1·0	6·0	0·43	0·007
C6a	♂	16·8	41·0	17·0	45·0	0·2	4·0	28·0	0·65	0·015	17·0	45·0	17·0	44·5*	nil	− 0·5	5·5†	0·37	0·008
C4a	♂	15·8	38·0	16·2	42·0	0·4	4·0	26·7	0·62	0·015			—		—		—	—	—
C5a	♂	14·7	28·0	14·8	29·5	0·1	1·5	25·5	0·59	0·021	14·8	29·5	14·8	29·5*	nil	nil	5·5†	0·37	0·013
A2a	♀	19·7	71·5	19·8	77·0	0·1	5·5	43·0	1·00	0·013	19·8	77·0	19·7	72·5	− 0·1	− 4·5	5·5	0·39	0·005
A1a	♀	18·8	60·0	18·9	60·0	0·1	nil	35·5	0·83	0·014	18·9	60·0	18·9	60·0	nil	nil	5·5	0·39	0·006
A3a	♀	17·8	57·5	17·9	60·0	0·1	2·5	44·0	1·02	0·017	17·9	60·0	17·9	58·0	nil	− 2·0	5·5	0·39	0·007
A6a	♀	16·4	41·0	16·9	45·0	0·5	4·0	26·0	0·60	0·014	16·9	45·0	16·9	46·0	nil	1·0	5·5	0·39	0·009
A4a	♀	16·3	38·0	16·8	42·0	0·5	4·0	27·0	0·63	0·016	16·8	42·0	16·8	42·0	nil	nil	5·5	0·39	0·009

* November 28th. † Period of 15 days.

TABLE 19.

SUMMARISED DATA CONCERNING MAXIMUM REQUIREMENTS AND GROWTH IN MALE AND FEMALE PLAICE.

Fish No.	Sex.	Period.	No. of days in period.	Initial size.		Final size.		Growth.		Total food per the period. (gm.).	Average weight of food per day for period. (gm.).
				Lth.	Wt.	Lth.	Wt.	Lth.	Wt.		
D1	♂	May 22–Oct. 29	160	12·2	14·0	15·9	40·5	3·7	26·5	420	2·6
D2	♂	May 22–Nov. 13	175	12·2	15·0	17·9	64·0	5·7	49·0	471	2·7
D3	♂	June 6–Nov. 28	175	12·0	15·5	21·0	105·0	9·0	89·5	585	3·3
D4	♂	June 6–Nov. 28	175	12·0	13·0	17·3	56·5	5·3	43·5	475	2·7
D5	♂	May 22–Nov. 28	190	11·9	14·5	15·8	38·0	3·9	23·5	417	2·2
D6	♂	May 22–Nov. 13	175	12·0	17·0	14·5	24·0	2·5	7·0	361	2·1
B1	♀	May 22–Nov. 27	189	13·2	24·0	21·0	105·5	7·8	81·5	618	3·3
B2	♀	May 22–Nov. 27	189	12·7	17·0	16·9	51·0	4·2	34·0	438	2·3
B3	♀	June 6–Nov. 27	174	12·6	18·0	14·5	32·0	1·9	14·0	379	2·2
B4	♀	May 22–Nov. 27	189	13·2	18·5	21·3	103·5	8·1	85·0	613	3·3
B5	♀	May 22–Sept. 16	117	12·3	15·0	13·3	20·0	1·0	5·0	190	1·6
B5a	♀	Sept. 17–Nov. 27	71	17·9	54·0	20·1	86·0	2·2	32·0	290	4·1
B6	♀	May 22–Nov. 27	189	12·0	16·0	16·7	51·0	4·7	35·0	448	2·4

TABLES 20–22.

Maximum Requirements and Growth of Male Plaice.

Table 20: D3.

Date. (1929)	Size of fish. Lth.	Wt.	Wt./Lth.³	Growth per period. Lth.	Wt.	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Cumulative growth. Lth.	Wt.	Total food to date from June 6 (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
June 6	12·0	15·5	0·009	—		—	—	—	—	—		—	—
,, 20	12·2	18·0	0·010	0·2	2·5	22·0	8·8	14	1·6	0·2	2·5	22	8·8
July 5	13·1	22·5	0·010	0·9	4·5	26·0	5·8	15	1·7	1·1	7·0	48	6·9
,, 19	14·1	30·0	0·011	1·0	7·5	36·0	4·8	14	2·6	2·1	14·5	84	5·8
Aug. 2	15·0	37·0	0·011	0·9	7·0	41·4	5·9	14	3·0	3·0	21·5	125	5·8
,, 16	15·9	41·5	0·010	0·9	4·5	44·2	9·8	14	3·1	3·9	26·0	170	6·5
,, 30	16·6	51·5	0·011	0·7	10·0	53·2	5·3	14	3·8	4·6	36·0	223	6·2
Sept. 16	17·8	65·5	0·012	1·2	14·0	68·5	4·9	17	4·0	5·8	50·0	291	5·8
Oct. 1	18·9	79·0	0·012	1·1	13·5	65·2	4·8	15	4·3	6·9	63·5	356	5·6
,, 15	19·7	90·0	0·012	0·8	11·0	76·5	7·0	14	5·5	7·7	74·5	433	5·8
,, 29	20·3	95·5	0·011	0·6	5·5	63·0	11·5	14	4·5	8·3	80·0	496	6·2
Nov. 13	20·7	103·0	0·012	0·4	7·5	50·0	6·7	15	3·3	8·7	87·5	546	6·2
,, 28	21·0	105·0	0·011	0·3	2·0	39·0	19·5	15	2·6	9·0	89·5	585	6·5

Table 21: D2.

May 22	12·2	15·0	0·008	—		—	—	—	—	—		—	—
June 6	12·6	19·5	0·010	0·4	4·5	40·0	8·9	15	2·7	0·4	4·5	40	8·9
,, 20	12·9	21·0	0·010	0·3	1·5	22·0	14·7	14	1·6	0·7	6·0	62	10·3
July 5	13·1	22·0	0·010	0·2	1·0	26·0	26·0	15	1·7	0·9	7·0	88	12·6
,, 19	13·9	27·0	0·010	0·8	5·0	33·9	6·8	14	2·4	1·7	12·0	122	10·2
Aug. 2	14·6	32·0	0·010	0·7	5·0	38·4	7·7	14	2·7	2·4	17·0	160	9·4
,, 16	15·3	32·5	0·009	0·7	0·5	43·1	86·2	14	3·1	3·1	17·5	203	11·6
,, 30	15·8	33·0	0·008	0·5	0·5	47·0	94·0	14	3·4	3·6	18·0	250	13·9
Sept. 16	16·6	49·0	0·010	0·8	16·0	58·4	3·6	17	3·4	4·4	34·0	309	9·1
Oct. 1	16·9	53·5	0·011	0·3	4·5	56·6	12·6	15	3·8	4·7	38·5	365	9·5
,, 15	17·4	58·0	0·011	0·5	4·5	42·5	9·4	14	3·0	5·2	43·0	408	9·5
,, 29	17·6	60·5	0·011	0·2	2·5	39·0	15·6	14	2·8	5·4	45·5	447	9·8
Nov. 13	17·9	64·0	0·011	0·3	3·5	24·0	6·9	15	1·6	5·7	49·0	471	9·6
,, 28	17·9	63·0	0·011	nil	−1·0	23·0−	—	15	1·5−	5·7	48·0	494	10·+

Table 22: D4.

June 6	12·0	13·0	0·008	—		—	—	—	—	—		—	—
,, 20	12·1	14·0	0·008	0·1	1·0	22·0	22·0	14	1·6	0·1	1·0	22	22·0
July 5	12·8	19·5	0·009	0·7	5·5	26·0	4·7	15	1·7	0·8	6·5	48	7·4
,, 19	13·5	24·0	0·010	0·7	4·5	33·5	7·4	14	2·4	1·5	11·0	81	7·4
Aug. 2	14·2	29·0	0·010	0·7	5·0	42·5	8·5	14	3·0	2·2	16·0	124	7·7
,, 16	14·7	32·5	0·010	0·5	3·5	42·0	12·0	14	3·0	2·7	19·5	166	8·5
,, 30	15·1	36·0	0·010	0·4	3·5	47·5	13·5	14	3·4	3·1	23·0	213	9·3
Sept. 16	15·6	40·0	0·011	0·5	4·0	59·9	15·0	17	4·0	3·6	27·0	273	10·1
Oct. 1	16·2	47·0	0·011	0·6	7·0	51·0	7·3	15	3·4	4·2	34·0	324	9·5
,, 15	16·7	51·0	0·011	0·5	4·0	51·5	12·9	14	3·7	4·7	38·0	376	9·9
,, 29	17·0	55·0	0·011	0·3	4·0	46·0	11·5	14	3·3	5·0	42·0	422	10·0
Nov. 13	17·3	57·0	0·011	0·3	2·0	30·5	15·2	15	2·0	5·3	44·0	452	10·3
,, 28	17·3	56·5	0·011	nil	−0·5	23−	—	15	1·5−	5·3	43·5	475−	10·9

TABLES 23–27.

Maximum Requirements and Growth of Female Plaice.

Table 23: B1.

Date. (1929)	Size of fish. Lth.	Wt.	Wt./Lth.³	Growth per period. Lth.	Wt.	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Cumulative growth. Lth.	Wt.	Total food to date from May 22 (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
May 22	13·2	24·0	0·010	—		—	—	—	—	—	—	—	—
June 6	14·0	31·0	0·011	0·8	7·0	40·0	5·7	15	2·7	0·8	7·0	40	5·7
,, 20	14·7	35·0	0·011	0·7	4·0	22·0	5·5	14	1·6	1·5	11·0	62	5·6
July 5	15·4	40·0	0·011	0·7	5·0	26·0	5·2	15	1·7	2·2	16·0	88	5·5
,, 19	16·2	47·0	0·011	0·8	7·0	38·0	5·4	14	2·7	3·0	23·0	126	5·5
Aug. 2	16·8	53·5	0·011	0·6	6·5	44·3	6·8	14	3·2	3·6	29·5	170	5·8
,, 16	17·5	61·0	0·011	0·7	7·5	46·2	6·2	14	3·3	4·3	37·0	216	5·8
,, 30	18·2	69·0	0·011	0·7	8·0	57·0	7·1	14	4·1	5·0	45·0	273	6·1
Sept. 16	19·0	79·5	0·012	0·8	10·5	69·0	6·6	17	4·1	5·8	55·5	342	6·2
Oct. 1	19·8	89·5	0·012	0·8	10·0	66·9	6·7	15	4·5	6·6	65·5	409	6·2
,, 15	20·2	96·0	0·012	0·4	6·5	63·0	9·7	14	4·5	7·0	72·0	472	6·6
,, 29	20·5	98·5	0·011	0·3	2·5	49·0	19·6	14	3·5	7·3	74·5	521	7·0
Nov. 13	20·8	104·0	0·012	0·3	5·5	54·0	9·8	15	3·6	7·6	80·0	575	7·2
,, 27	21·0	105·5	0·011	0·2	1·5	43–	28·7	14	3·1–	7·8	81·5	618	7·6
Dec. 11	21·0	105·5	0·011	nil	nil	28·5–	—	14	2·0–	7·8	81·5	646	—

Table 24: B4.

May 22	13·2	18·5	0·008	—		—	—	—	—	—	—	—	—
June 6	13·9	26·0	0·009	0·7	7·5	40·0	5·3	15	2·7	0·7	7·5	40	5·3
,, 20	14·7	32·0	0·010	0·8	6·0	22·0	3·6	14	1·6	1·5	13·5	62	4·6
July 5	15·3	36·5	0·010	0·6	4·5	26·0	5·8	15	1·7	2·1	18·0	88	4·9
,, 19	16·1	43·0	0·010	0·8	6·5	36·5	5·6	14	2·6	2·9	24·5	124	5·1
Aug. 2	16·9	50·5	0·010	0·8	7·5	48·7	6·5	14	3·5	3·7	32·0	173	5·4
,, 16	17·7	60·5	0·011	0·8	10·0	51·4	5·1	14	3·7	4·5	42·0	224	5·3
,, 30	18·4	66·0	0·011	0·7	5·5	54·3	9·9	14	3·9	5·2	47·5	279	5·9
Sept. 16	19·0	76·0	0·011	0·6	10·0	65·5	6·5	17	3·9	5·8	57·5	344	6·0
Oct. 1	19·6	83·5	0·011	0·6	7·5	55·2	7·4	15	3·7	6·4	65·0	400	6·2
,, 15	20·2	90·0	0·011	0·6	6·5	59·0	9·1	14	4·2	7·0	71·5	459	6·4
,, 29	20·7	102·0	0·011	0·5	12·0	63·0	5·2	14	4·5	7·5	83·5	522	6·3
Nov. 13	21·2	108·0	0·011	0·5	6·0	52·5	8·7	15	3·5	8·0	89·5	574	6·4
,, 27	21·3	103·5	0·011	0·1	–4·5	38·5–	—	14	2·7	8·1	85·0	613	7·2

Table 25: B5a.

Sept. 17	17·9	54·0	0·009	—		—	—	—	—	—	—	—	—
Oct. 1	18·1	59·0	0·010	0·2	5·0	42·2	8·4	14	3·0	0·2	5·0	42	8·4
,, 15	18·9	72·5	0·011	0·8	13·5	77·5	5·7	14	5·5	1·0	18·5	120	6·5
,, 29	19·7	86·0	0·011	0·8	13·5	76·5	5·7	14	5·5	1·8	32·0	196	6·1
Nov. 13	20·1	89·0	0·011	0·4	3·0	57·5	19·2	15	3·8	2·2	35·0	254	7·3
,, 27	20·1	86·0	0·011	nil	–3·0	37–	—	14	2·6	2·2	32·0	291–	9·1

Table 26: B2.

Date. (1929)	Size of fish. Lth.	Wt.	Wt./Lth.³	Growth per period. Lth.	Wt.	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Cumulative growth. Lth.	Wt.	Total food to date from May 22 (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
May 22	12·7	17·0	0·008	—		—	—	—	—	—	—	—	—
June 6	13·3	23·0	0·010	0·6	6·0	40·0	6·7	15	2·7	0·6	6·0	40	6·7
„ 20	14·0	27·0	0·010	0·7	4·0	22·0	5·5	14	1·6	1·3	10·0	62	6·2
July 5	14·2	27·0	0·009	0·2	nil	26·0	—	15	1·7	1·5	10·0	88	8·8
„ 19	14·5	28·5	0·009	0·3	1·5	23·0	15·3	14	1·6	1·8	11·5	111	9·7
Aug. 2	14·8	30·0	0·009	0·3	1·5	30·2	20·1	14	2·2	2·1	13·0	141	10·8
„ 16	14·9	30·5	0·009	0·1	0·5	29·7	59·4	14	2·1	2·2	13·5	171	12·7
„ 30	15·2	34·0	0·010	0·3	3·5	37·8	10·8	14	2·7	2·5	17·0	209	12·3
Sept. 16	15·5	36·5	0·010	0·3	2·5	45·6	18·2	17	2·7	2·8	19·5	254	13·0
Oct. 1	15·7	39·5	0·010	0·2	3·0	45·3	15·1	15	3·0	3·0	22·5	300	13·3
„ 15	15·8	40·0	0·010	0·1	0·5	42·0	84·0	14	3·0	3·1	23·0	342	14·9
„ 29	16·3	45·0	0·010	0·5	5·0	42·5	8·5	14	3·0	3·6	28·0	384	13·7
Nov. 13	16·7	49·5	0·011	0·4	4·5	29·5	6·6	15	2·0	4·0	32·5	414	12·7
„ 27	16·9	51·0	0·011	0·2	1·5	24·5 –	16·3	14	1·7	4·2	34·0	438 –	12·9

Table 27: B6.

May 22	12·0	16·0	0·009	—		—	—	—	—	—	—	—	—
June 6	12·7	23·0	0·011	0·7	7·0	40·0	5·7	15	2·7	0·7	7·0	40	5·7
„ 20	13·3	25·0	0·011	0·6	2·0	22·0	11·0	14	1·6	1·3	9·0	62	6·8
July 5	13·8	28·0	0·011	0·5	3·0	26·0	8·7	15	1·7	1·8	12·0	88	7·3
„ 19	14·5	29·0	0·010	0·7	1·0	34·0	34·0	14	2·4	2·5	13·0	122	9·4
Aug. 2	15·1	36·0	0·010	0·6	7·0	31·6	4·5	14	2·3	3·1	20·0	154	7·7
„ 16	15·2	37·0	0·011	0·1	1·0	34·0	34·0	14	2·4	3·2	21·0	188	9·0
„ 30	15·4	38·5	0·011	0·2	1·5	36·5	24·3	14	2·6	3·4	22·5	224	10·0
Sept. 16	15·7	41·5	0·011	0·3	3·0	47·1	15·7	17	2·8	3·7	25·5	271	10·6
Oct. 1	15·9	44·0	0·011	0·2	2·5	44·0	17·6	15	2·9	3·9	28·0	315	11·2
„ 15	16·4	49·0	0·011	0·5	5·0	47·5	9·5	14	3·4	4·4	33·0	363	11·0
„ 29	16·5	50·0	0·011	0·1	1·0	38·5	38·5	14	2·7	4·5	34·0	401	11·8
Nov. 13	16·7	53·5	0·011	0·2	3·5	23·0	6·6	15	1·5	4·7	37·5	424	11·3
„ 27	16·7	51·0	0·011	nil	– 2·5	24 –	—	14	1·7 –	4·7	35·0	448	12·8

TABLES 28–31.

Maintenance Requirements of Male Plaice.

Table 28: L1.

Date. (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average wt. of food per day for each period. (gm.)	Total food to date. (gm.)	No. of days since commencement of expt.	Average wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
Aug. 22	17·0	50·3	—		—	—	—	—	—	—	—	—
Sept. 2	17·0	49·0	nil	– 1·3	8·2	11	0·74	8·2	11	0·74	nil	– 1·3
„ 16	17·0	47·7	nil	– 1·3	11·9	14	0·85	20·1	25	0·80	nil	– 2·6
„ 30	17·0	49·9	nil	2·2	13·3	14	0·95	33·4	39	0·86	nil	– 0·4
Oct. 14	17·2	53·1	0·2	3·2	13·4	14	0·96	46·8	53	0·88	0·2	2·8
„ 28	17·6	52·7	0·4	– 0·4	12·7	14	0·91	59·5	67	0·89	0·6	2·4
Nov. 11	17·6	54·5	nil	1·8	13·2	14	0·94	72·7	81	0·90	0·6	4·2
„ 25	17·6	54·1	nil	– 0·4	12·6	14	0·90	85·3	95	0·90	0·6	3·8
Dec. 9	17·6	54·4	nil	0·3	8·3	14	0·59	93·6	109	0·86	0·6	4·1
„ 23	17·6	53·7	nil	– 0·7	10·5	14	0·75	104·1	123	0·85	0·6	3·4

Table 29: L4a above, L4b below.

Date. (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average wt. of food per day for each period. (gm.)	Total food to date. (gm.)	No. of days since commencement of expt.	Average wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
Aug. 19	20·8	84·7	—		—	—	—	—	—	—	—	
Sept. 2	20·8	83·0	nil	− 1·7	11·9	14	0·85	11·9	14	0·85	nil	− 1·7
„ 16	20·8	83·7	nil	0·7	12·3	14	0·88	24·2	28	0·86	nil	− 1·0
Sept. 30	20·8	75·1	—		—	—	—	—	—	—	—	
Oct. 14	20·9	77·7	0·1	2·6	14·0	14	1·00	14·0	14	1·0	0·1	2·6
„ 28	20·9	75·1	nil	− 2·6	10·6	14	0·76	24·6	28	0·88	0·1	nil
Nov. 11	21·0	77·2	0·1	2·1	19·6	14	1·40	44·2	42	1·05	0·2	2·1
„ 25	21·0	76·9	nil	− 0·3	18·2	14	1·30	62·4	56	1·11	0·2	1·8
Dec. 9	21·0	77·2	nil	0·3	11·9	14	0·85	74·3	70	1·06	0·2	2·1
„ 23	21·0	78·0	nil	0·8	16·2	14	1·16	90·5	84	1·08	0·2	2·9

Table 30: L3.

Sept. 8	21·4	105·3	—		—	—	—	—	—	—	—	
„ 16	21·4	102·5	nil	− 2·8	10·4	8	1·30	10·4	8	1·30	nil	− 2·8
„ 30	21·5	104·2	0·1	1·7	21·1	14	1·51	31·5	22	1·43	0·1	− 1·1
Oct. 14	21·6	108·2	0·1	4·0	21·1	14	1·51	52·6	36	1·46	0·2	2·9
„ 28	21·7	106·0	0·1	− 2·2	16·5	14	1·16	69·1	50	1·38	0·3	0·7
Nov. 11	22·0	110·2	0·3	4·2	19·4	14	1·39	88·5	64	1·38	0·6	4·9
„ 25	22·0	109·4	nil	− 0·8	14·8	14	1·06	103·3	78	1·32	0·6	4·1
Dec. 9	22·0	110·4	nil	1·0	12·1	14	0·86	115·4	92	1·25	0·6	5·1
„ 23	22·1	111·9	0·1	1·5	18·9	14	1·35	134·3	106	1·27	0·7	6·6

Table 31: L2.

Aug. 19	22·8	129·9	—		—	—	—	—	—	—	—	
Sept. 2	22·8	129·1	nil	− 0·8	17·5	14	1·25	17·5	14	1·25	nil	− 0·8
„ 16	22·8	123·3	nil	− 5·8	17·6	14	1·26	35·1	28	1·25	nil	− 6·6
„ 30	22·8	126·7	nil	3·4	23·4	14	1·67	58·5	42	1·39	nil	− 3·2
Oct. 14	22·9	129·7	0·1	3·0	23·5	14	1·68	82·0	56	1·46	0·1	− 0·2
„ 28	23·0	123·2	0·1	− 6·5	16·8	14	1·20	98·8	70	1·41	0·2	− 6·7
Nov. 11	23·0	130·5	nil	7·3	24·3	14	1·74	123·1	84	1·47	0·2	0·6
„ 25	23·1	129·4	0·1	− 1·1	18·1	14	1·29	141·2	98	1·44	0·3	− 0·5
Dec. 9	23·1	130·7	nil	1·3	12·9	14	0·92	154·1	112	1·38	0·3	0·8
„ 23	23·1	130·9	nil	0·2	20·4	14	1·46	174·5	126	1·38	0·3	1·0

TABLE 32.

Maintenance in the Male Plaice, L2a and L1a.

Fish No.	Date. (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day during each period. (gm.)	Total food to date. (gm.)	No. of days since commencement of exp.	Average weight of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
	July 8	15·2	22·3	—		—	—	—	—	—	—	—	
L2a	„ 22	15·2	21·1	nil	− 1·2	9·8	14	0·70	9·8	14	0·70	nil	− 1·2
	Aug. 5	15·2	20·9	nil	− 0·2	10·2	14	0·73	20·0	28	0·71	nil	− 1·4
	July 8	21·2	62·5	—		—	—	—	—	—	—	—	
L1a	„ 22	21·2	61·0	nil	− 1·5	9·4	14	0·67	9·4	14	0·67	nil	− 1·5
	Aug. 5	21·2	61·0	nil	nil	10·2	14	0·73	19·6	28	0·70	nil	− 1·5
	„ 19	21·2	61·5	nil	0·5	11·2	14	0·80	30·8	42	0·73	nil	− 1·0

Fish No.	Lth.	Wt.	Period.	No. of days in period.	Increase in weight per period (gm.)	Average weight of food per day (gm.)	Food per day / average body weight (for period).
L2a	22·3	20·9	July 8th to Aug. 5th	28	− 1·4	0·71	0·033
L1a	62·5	61·5	July 8th to Aug. 19th	42	− 1·0	0·73	0·012

TABLE 33.

Maintenance in the Male Plaice, L1, L4B, L3, and L2.

		50·3	**L1**	53·7	75·1	**L4B**	78·0	105·3	**L3**	111·9	129·9	**L2**	130·9
Date. (1929)	No. of days in period.	Increase in weight per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).	Increase in weight per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).	Increase in weight per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).	Increase in weight per period. (gm.)	Food per day/ /average body-wt. (for period).	Food per day/ /mid body-wt. (to date).
Aug. 19*	—	—	—	—	—	—	—	—	—	—	—	—	—
Sept. 2†	14	− 1·3	0·015	0·015	—	—	—	—	—	—	− 0·8	0·010	0·010
„ 16	14	− 1·3	0·018	0·016	—	—	—	− 2·8	0·013	0·013	− 5·8	0·010	0·010
„ 30	14	2·2	0·019	0·017	—	—	—	1·7	0·015	0·014	3·4	0·013	0·011
Oct. 14	14	3·2	0·019	0·017	2·6	0·013	0·013	4·0	0·014	0·014	3·0	0·013	0·011
„ 28	14	− 0·4	0·017	0·017	− 2·6	0·010	0·012	− 2·2	0·011	0·013	− 6·5	0·009	0·011
Nov. 11	14	1·8	0·018	0·017	2·1	0·018	0·014	4·2	0·013	0·013	7·3	0·014	0·011
„ 25	14	− 0·4	0·017	0·017	− 0·3	0·017	0·015	− 0·8	0·010	0·012	− 1·1	0·010	0·011
Dec. 9	14	0·3	0·011	0·016	0·3	0·011	0·014	1·0	0·008	0·013	1·3	0·007	0·011
„ 23	14	− 0·7	0·014	0·016	0·8	0·015	0·014	1·5	0·012	0·012	0·2	0·011	0·011

* August 22nd for L1. † September 8th for L3.

TABLES 34–37.

Maintenance in Female Plaice.

Table 34: L5.

Date. (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average wt. of food per day for each period. (gm.)	Total food to date. (gm.)	No. of days since commencement of expt.	Average wt. of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
Aug. 8	18·5	62·8	—		—	—	—	—	—	—	—	
,, 19	18·5	60·5	nil	− 2·3	8·8	11	0·80	8·8	11	0·80	nil	− 2·3
Sept. 2	18·5	59·3	nil	− 1·2	11·8	14	0·84	20·6	25	0·82	nil	− 3·5
,, 16	—		—		13·2	14	0·94	33·8	39	0·87	—	
,, 30	18·4	62·2	− 0·1	2·9	13·2	14	0·94	47·0	53	0·89	− 0·1	− 0·6
Oct. 14	18·4	65·7	nil	3·5	15·3	14	1·09	62·3	67	0·93	− 0·1	2·9
,, 28	18·5	65·3	0·1	− 0·4	11·1	14	0·79	73·4	81	0·91	nil	2·5
Nov. 11	18·6	65·6	0·1	0·3	11·3	14	0·81	84·7	95	0·89	0·1	2·8
,, 25	18·6	66·9	nil	1·3	11·3	14	0·81	96·0	109	0·88	0·1	4·1
Dec. 9	18·7	67·0	0·1	0·1	6·2	14	0·44	102·2	123	0·83	0·2	4·2
,, 23	18·7	67·5	nil	0·5	10·4	14	0·74	112·6	137	0·82	0·2	4·7

Table 35: L6.

Sept. 16	19·2	71·2	—		—	—	—	—	—	—	—	
,, 30	19·2	70·5	nil	− 0·7	15·4	14	1·10	15·4	14	1·10	nil	− 0·7
Oct. 14	19·2	73·5	nil	3·0	17·8	14	1·27	33·2	28	1·19	nil	2·3
,, 28	19·2	70·3	nil	− 3·2	13·3	14	0·95	46·5	42	1·11	nil	− 0·9
Nov. 11	19·2	71·7	nil	1·4	17·3	14	1·24	63·8	56	1·14	nil	0·5
,, 25	19·3	70·1	0·1	− 1·6	19·6	14	1·40	83·4	70	1·19	0·1	− 1·1
Dec. 9	19·3	69·9	nil	− 0·2	11·0	14	0·79	94·4	84	1·12	0·1	− 1·3
,, 23	19·3	70·4	nil	0·5	18·0	14	1·29	112·4	98	1·15	0·1	− 0·8

Table 36: L7.

Sept. 4	22·3	104·8	—		—	—	—	—	—	—	—	
,, 16	22·3	100·1	nil	− 4·7	15·6	12	1·30	15·6	12	1·30	nil	− 4·7
,, 30	22·4	97·5	0·1	− 2·6	18·2	14	1·30	33·8	26	1·30	0·1	− 7·3
Oct. 14	22·4	99·2	nil	1·7	19·6	14	1·40	53·4	40	1·33	0·1	− 5·6
,, 28	22·4	94·4	nil	− 4·8	16·4	14	1·17	69·8	54	1·29	0·1	− 10·4
Nov. 11	22·5	99·5	0·1	5·1	20·5	14	1·46	90·3	68	1·33	0·2	− 5·3
,, 25	22·7	100·6	0·2	1·1	17·7	14	1·26	108·0	82	1·32	0·4	− 4·2
Dec. 9	22·7	99·8	nil	− 0·8	10·6	14	0·76	118·6	96	1·24	0·4	− 5·0
,, 23	22·7	100·0	nil	0·2	16·6	14	1·19	135·2	110	1·23	0·4	− 4·8

Table 37: L8.

Aug. 22	21·9	102·0	—		—	—	—	—	—	—	—	
Sept. 2	22·0	102·1	0·1	0·1	14·3	11	1·30	14·3	11	1·30	0·1	0·1
,, 16	22·0	102·8	nil	0·7	18·2	14	1·30	32·5	25	1·30	0·1	0·8
,, 30	22·1	104·5	0·1	1·7	19·0	14	1·36	51·5	39	1·32	0·2	2·5
Oct. 16	23·0	114·0	—		—	—	—	—	—	—	—	
,, 28	23·1	111·3	0·1	− 2·7	16·8	12	1·40	16·8	12	1·40	0·1	− 2·7
Nov. 11	23·2	114·0	0·1	2·7	23·4	14	1·67	40·2	26	1·55	0·2	nil
,, 25	23·3	113·6	0·1	− 0·4	20·2	14	1·44	60·4	40	1·51	0·3	− 0·4
Dec. 9	23·3	114·2	nil	0·6	13·4	14	0·96	73·8	54	1·37	0·3	0·2
,, 23	23·3	116·5	nil	2·3	20·8	14	1·49	94·6	68	1·39	0·3	2·5

TABLE 38.

Maintenance in the Female Plaice, L5, L6, L7, and L8 (a & b).

Date. (1929)	No. of days in period.	62·8 Increase in weight per period. (gm.)	L5 Food per day/ /average body-wt. (for period).	67·5 Food per day/ /mid body-wt. (to date).	71·2 Increase in weight per period. (gm.)	L6 Food per day/ /average body-wt. (for period).	70·4 Food per day/ /mid body-wt. (to date).	104·8 Increase in weight per period. (gm.)	L7 Food per day/ /average body-wt. (for period).	100·0 Food per day/ /mid body-wt. (to date).	102·0 / 114·0 Increase in weight per period. (gm.)	$L8^{a}_{b}$ Food per day/ /average body-wt. (for period).	104·5 / 116·5 Food per day/ /mid body-wt. (to date).
Aug. 8	—				—			—			—		
„ 19†	11	− 2·3	0·013	0·013							—		
Sept. 2*	14	− 1·2	0·014	0·013				—			0·1	0·013	0·013
„ 16	14	—	—	—	—			− 4·7	0·013	0·013	0·7	0·013	0·013
„ 30	14	2·9	0·015	0·014	− 0·7	0·016	0·016	− 2·6	0·013	0·013	1·7	0·013	0·013
Oct. 14‡	14	3·5	0·017	0·014	3·0	0·018	0·016	1·7	0·014	0·013	—		
„ 28	14	− 0·4	0·012	0·014	− 3·2	0·013	0·016	− 4·8	0·012	0·013	− 2·7	0·012	0·012
Nov. 11	14	0·3	0·012	0·014	1·4	0·017	0·016	5·1	0·015	0·013	2·7	0·015	0·014
„ 25	14	1·3	0·012	0·014	− 1·6	0·020	0·017	1·1	0·013	0·013	− 0·4	0·013	0·013
Dec. 9	14	0·1	0·007	0·013	− 0·2	0·011	0·016	− 0·8	0·008	0·012	0·6	0·008	0·012
„ 23	14	0·5	0·011	0·013	0·5	0·018	0·016	0·2	0·012	0·012	2·3	0·013	0·012

* September 4th for L7. † August 22nd for L8. ‡ October 16th for L8.

TABLE 39.

Maintenance in the Female Plaice, L6a, L5a, L8c, and L7a.

Fish No.	Date. (1929)	Size of fish. Lth.	Wt.	Growth per period. Lth.	Wt.	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day for each period. (gm.)	Total food to date. (gm.)	No. of days since commencement of exp.	Average weight of food per day to date. (gm.)	Cumulative growth. Lth.	Wt.
	June 24	15·2	22·9	—		—	—	—	—	—	—	—	
L6a	July 8	15·2	21·7	nil	−1·2	7·6	14	0·54	7·6	14	0·54	nil	−1·2
	,, 22	15·3	21·8	0·1	0·1	9·1	14	0·65	16·7	28	0·60	0·1	−1·1
	June 24	17·0	35·5	—		—	—	—	—	—	—	—	
L5a	July 8	17·1	34·0	0·1	−1·5	8·1	14	0·58	8·1	14	0·58	0·1	−1·5
	,, 22	17·1	33·7	nil	−0·3	9·0	14	0·64	17·1	28	0·61	0·1	−1·8
	July 8	18·5	40·9	—		—	—	—	—	—	—	—	
L8c	,, 22	18·5	37·3	nil	−3·6	9·4	14	0·67	9·4	14	0·67	nil	−3·6
	Aug. 5	18·6	37·9	0·1	0·6	11·2	14	0·80	20·6	28	0·74	0·1	−3·0
	,, 19	18·6	38·0	nil	0·1	11·0	14	0·79	31·6	42	0·75	0·1	−2·9
	July 22	19·7	57·8	—		—	—	—	—	—	—	—	
L7a	Aug. 5	19·7	57·0	nil	−0·8	10·9	14	0·78	10·9	14	0·78	nil	−0·8
	,, 19	19·7	54·9	nil	−2·1	10·9	14	0·78	21·8	28	0·78	nil	−2·9
	Sept. 2	19·7	55·2	nil	0·3	11·7	14	0·84	33·5	42	0·80	nil	−2·6

TABLE 40.

Maintenance in the Female Plaice, L6a, L5a, L8c, and L7a.

Fish No.	Period.	No. of days in period.	Initial weight. (gm.)	Final weight. (gm.)	Increase in weight. (gm.)	Food per period. (gm.)	Average weight of food per day. (gm.)	Food per day/ /average body-wt. (for period).
L6a	June 24–July 22	28	22·9	21·8	−1·1	16·7	0·60	0·027
L5a	,, ,,	28	35·5	33·7	−1·8	17·1	0·61	0·018
L8c	July 22–Aug. 19	28	37·3	38·0	0·7	22·2	0·79	0·021
L7a	July 22–Sept. 2	42	57·8	55·2	−2·6	33·5	0·80	0·014

TABLE 41.

Maximum Requirements and Growth.

Fish No.	Sex.	Period. (1929)	No. of days in period.	Initial size.		Final size.		Growth.		Total food per the period. (gm.)	Average weight of food per day for the period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.
				Lth.	Wt.	Lth.	Wt.	Lth.	Wt.			
L11	♂	June 24–Nov. 25	154	16·1	23·2	23·1	157·4	7·0	134·2	953	6·2	7·1
L10	♂	June 24–Sept. 30	98	16·6	36·2	19·1	82·8	2·5	46·6	536	5·5	11·5
L12	♂	June 24–Nov. 25	154	18·1	44·0	21·8	111·4	3·7	67·4	758	4·9	11·2
L9	♂	June 24–Sept. 30	98	19·7	57·5	21·3	102·6	1·6	45·1	676	6·9	15·0
L13	♀	June 24–Nov. 25	154	15·1	27·0	19·2	70·4	4·1	43·4	626	4·1	14·4
L15	♀	June 24–Nov. 25	154	15·1	31·9	23·0	140·7	7·9	108·8	883	5·7	8·1
L16	♀	June 24–Nov. 25	154	18·4	50·5	24·1	175·0	5·7	124·5	1060	6·9	8·5
L14	♀	July 15–Nov. 25	133	23·0	90·1	25·7	184·8	2·7	94·7	905	6·8	9·6

TABLES 42–45.

Maximum Requirements and Growth of Male Plaice.

Table 42 : L11.

Date. (1929)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Cumulative growth. Lth.	Cumulative growth. Wt.	Total food to date. (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
June 24	16·1	23·2	0·006	—		—	—	—	—	—		—	—
July 8	17·4	48·5	0·009	1·3	25·3	57·2	2·3	14	4·1	1·3	25·3	57	2·3
,, 22	17·8	61·2	0·011	0·4	12·7	85·0	6·7	14	6·1	1·7	38·0	142	3·7
Aug. 5	18·2	66·7	0·011	0·4	5·5	98·0	17·8	14	7·0	2·1	43·5	240	5·5
,, 19	19·2	83·1	0·012	1·0	16·4	100·4	6·1	14	7·2	3·1	59·9	340	5·7
Sept. 2	20·0	97·1	0·012	0·8	14·0	102·3	7·3	14	7·3	3·9	73·9	443	6·0
,, 16	20·4	106·0	0·012	0·4	8·9	102·0	11·5	14	7·3	4·3	82·8	545	6·6
,, 30	21·3	122·8	0·013	0·9	16·8	104·4	6·2	14	7·5	5·2	99·6	649	6·5
Oct. 14	21·8	138·0	0·013	0·5	15·2	105·3	6·9	14	7·5	5·7	114·8	755	6·6
,, 28	22·8	149·2	0·013	1·0	11·2	92·0	8·2	14	6·6	6·7	126·0	847	6·7
Nov. 11	23·1	158·6	0·013	0·3	9·4	79·1	8·4	14	5·6	7·0	135·4	926	6·8
,, 25	23·1	157·4	0·013	nil	−1·2	26·6	—	14	1·9	7·0	134·2	953	7·1
Dec. 9	23·1	151·6	0·012	nil	−5·8	4·6	—	14	0·3	7·0	128·4	958	7·5
,, 23	23·1	147·2	0·012	nil	−4·4	5·9	—	14	0·4	7·0	124·0	963	7·8

Table 43 : L10.

June 24	16·6	36·2	0·008	—		—	—	—	—	—	—	—	—
July 8	16·9	42·8	0·009	0·3	6·6	52·2	7·9	14	3·7	0·3	6·6	52	7·9
,, 22	17·4	52·8	0·010	0·5	10·0	69·7	7·0	14	5·0	0·8	16·6	122	7·3
Aug. 5	17·9	62·0	0·011	0·5	9·2	84·0	9·1	14	6·0	1·3	25·8	206	8·0
,, 19	18·4	70·3	0·011	0·5	8·3	90·7	10·9	14	6·5	1·8	34·1	297	8·7
Sept. 2	18·7	77·5	0·012	0·3	7·2	94·0	13·1	14	6·7	2·1	41·3	391	9·5
,, 16	18·8	76·2	0·011	0·1	−1·3	77·6	—	14	5·5	2·2	40·0	468	11·7
,, 30	19·1	82·8	0·012	0·3	6·6	67·9	10·3	14	4·8	2·5	46·6	536	11·5

Table 44. : L9.

June 24	19·7	57·5	0·008	—		—	—	—	—	—	—	—	—
July 8	19·8	59·0	0·008	0·1	1·5	58·2	38·8	14	4·2	0·1	1·5	58	38·8
,, 22	20·0	68·9	0·009	0·2	9·9	82·5	8·3	14	5·9	0·3	11·4	141	12·4
Aug. 5	20·4	79·8	0·009	0·4	10·9	102·3	9·4	14	7·3	0·7	22·3	243	10·4
,, 19	20·9	93·6	0·010	0·5	13·8	102·7	7·4	14	7·3	1·2	36·1	346	9·6
Sept. 2	21·2	98·3	0·010	0·3	4·7	110·0	23·4	14	7·9	1·5	40·8	456	11·2
,, 16	21·3	99·4	0·010	0·1	1·1	107·1	97·4	14	7·0	1·6	41·9	563	13·4
,, 30	21·3	102·6	0·011	nil	3·2	113·1	35·3	14	2·5	1·6	45·1	676	15·0

Table 45 : L12.

June 24	18·1	44·0	0·007	—		—	—	—	—	—	—	—	—
July 8	18·4	50·3	0·008	0·3	6·3	57·7	9·2	14	4·1	0·3	6·3	58	9·2
,, 22	18·5	58·7	0·009	0·1	8·4	73·0	8·7	14	5·2	0·4	14·7	131	8·9
Aug. 5	18·8	62·7	0·009	0·3	4·0	76·3	19·1	14	5·4	0·7	18·7	207	11·1
,, 19	19·3	73·3	0·010	0·5	10·6	78·6	7·4	14	5·6	1·2	29·3	286	9·8
Sept. 2	19·8	81·6	0·011	0·5	8·3	82·8	10·0	14	5·9	1·7	37·6	368	9·8
,, 16	20·1	87·1	0·011	0·3	5·5	83·7	15·2	14	6·0	2·0	43·1	452	10·5
,, 30	20·7	102·5	0·012	0·6	15·4	86·4	5·6	14	6·2	2·6	58·5	538	9·2
Oct. 14	21·2	109·0	0·011	0·5	6·5	87·2	13·4	14	6·2	3·1	65·0	626	9·6
,, 28	21·7	111·7	0·011	0·5	2·7	73·8	27·3	14	5·3	3·6	67·7	699	10·3
Nov. 11	21·8	114·7	0·011	0·1	3·0	50·0	16·7	14	3·6	3·7	70·7	749	10·6
,, 25	21·8	111·4	0·011	nil	−3·3	8·4	—	14	0·6	3·7	67·4	758	11·2
Dec. 9	21·9	108·7	0·010	0·1	−2·7	1·9	—	14	0·1	3·8	64·7	760	11·7
,, 23	22·0	104·5	0·010	0·1	−4·2	2·7	—	14	0·2	3·9	60·5	763	12·6

TABLES 46–49.

Maximum Requirements and Growth of Female Plaice.

Table 46: L15.

Date. (1929)	Size of fish. Lth.	Wt.	Wt./Lth.³	Growth per period. Lth.	Wt.	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Food per day during each period. (gm.)	Cumulative growth. Lth.	Wt.	Total food to date from June 24 (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
June 24	15·1	31·9	0·009	—		—	—	—	—	—		—	—
July 8	16·8	38·1	0·008	1·7	6·2	55·7	9·0	14	4·0	1·7	6·2	56	9·0
,, 22	17·4	49·5	0·009	0·6	11·4	77·2	6·8	14	5·5	2·3	17·6	133	7·6
Aug. 5	17·9	57·5	0·010	0·5	8·0	91·0	11·4	14	6·5	2·8	25·6	224	8·7
,, 19	18·8	72·2	0·011	0·9	14·7	91·2	6·2	14	6·5	3·7	40·3	315	7·8
Sept. 2	19·7	85·5	0·011	0·9	13·3	97·6	7·3	14	7·0	4·6	53·6	413	7·7
,, 16	20·2	91·8	0·011	0·5	6·3	95·4	15·1	14	6·8	5·1	59·9	507	8·5
,, 30	21·1	112·5	0·012	0·9	20·7	101·1	4·9	14	7·2	6·0	80·6	608	7·5
Oct. 14	22·1	123·8	0·011	1·0	11·3	101·3	9·0	14	7·2	7·0	91·9	709	7·7
,, 28	22·7	137·6	0·012	0·6	13·8	83·2	6·0	14	5·9	7·6	105·7	792	7·5
Nov. 11	23·0	141·1	0·012	0·3	3·5	65·4	18·4	14	4·7	7·9	109·2	858	7·9
,, 25	23·0	140·7	0·012	nil	−0·4	24·8	—	14	1·8	7·9	108·8	883	8·1
Dec. 9	23·0	137·0	0·011	nil	−3·7	4·6	—	14	0·3	7·9	105·1	888	8·4
,, 23	23·0	135·5	0·011	nil	−1·5	8·0	—	14	0·6	7·9	103·6	896	8·6

Table 47.: L16.

June 24	18·4	50·5	0·008	—		—	—	—	—	—		—	—
July 8	18·8	61·1	0·009	0·4	10·6	61·2	5·8	14	4·4	0·4	10·6	61	5·8
,, 22	19·1	70·5	0·010	0·3	9·4	81·5	8·6	14	5·8	0·7	20·0	143	7·1
Aug. 5	19·6	83·4	0·011	0·5	12·9	102·2	7·9	14	7·3	1·2	32·9	245	7·4
,, 19	20·5	99·9	0·012	0·9	16·5	109·5	6·6	14	7·8	2·1	49·4	354	7·2
Sept. 2	21·3	117·5	0·012	0·8	17·6	118·5	6·7	14	8·5	2·9	67·0	473	7·1
,, 16	21·7	130·5	0·013	0·4	13·0	122·3	9·4	14	8·7	3·3	80·0	595	7·4
,, 30	22·5	147·1	0·013	0·8	16·6	124·0	7·5	14	8·9	4·1	96·6	719	7·4
Oct. 14	23·0	169·1	0·014	0·5	22·0	124·7	5·7	14	8·9	4·6	118·6	844	7·1
,, 28	23·7	179·6	0·014	0·7	10·5	107·6	10·2	14	7·7	5·3	129·1	951	7·4
Nov. 11	24·0	179·3	0·013	0·3	−0·3	81·9	—	14	5·8	5·6	128·8	1033	8·0
,, 25	24·1	175·0	0·012	0·1	−4·3	26·6	—	14	1·9	5·7	124·5	1060	8·5
Dec. 9	24·1	171·1	0·012	nil	−3·9	6·4	—	14	0·5	5·7	120·6	1066	8·8
,, 23	24·1	170·8	0·012	nil	−0·3	10·6	—	14	0·8	5·7	120·3	1077	8·9

Table 48: L13.

June 24	15·1	27·0	0·008	—		—	—	—	—	—		—	—
July 8	15·5	31·1	0·008	0·4	4·1	36·7	9·0	14	2·6	0·4	4·1	37	9·0
,, 22	15·9	40·6	0·010	0·4	9·5	70·0	7·4	14	5·0	0·8	13·6	107	7·9
Aug. 5	16·6	48·1	0·011	0·7	7·5	81·4	10·9	14	5·8	1·5	21·1	188	8·9
,, 19	17·3	55·6	0·011	0·7	7·5	68·8	9·2	14	4·9	2·2	28·6	257	9·0
Sept. 2	17·8	61·9	0·011	0·5	6·3	67·6	10·7	14	4·8	2·7	34·9	324	9·3
,, 16	18·5	64·0	0·010	0·7	2·1	69·9	33·3	14	5·0	3·4	37·0	394	10·6
,, 30	18·7	69·0	0·011	0·2	5·0	70·3	14·1	14	5·0	3·6	42·0	464	11·0
Oct. 14	19·0	71·8	0·010	0·3	2·8	70·0	25·0	14	5·0	3·9	44·8	534	11·9
,, 28	19·2	71·4	0·010	0·2	−0·4	48·4	—	14	3·5	4·1	44·4	583	13·1
Nov. 11	19·2	71·0	0·010	nil	−0·4	36·3	—	14	2·6	4·1	44·0	619	14·1
,, 25	19·2	70·4	0·010	nil	−0·6	7·2	—	14	0·5	4·1	43·4	626	14·4
Dec. 9	19·2	69·1	0·010	nil	−1·3	3·7	—	14	0·3	4·1	42·1	630	15·0
,, 23	19·2	68·5	0·010	nil	−0·6	5·1	—	14	0·4	4·1	41·5	635	15·3

Table 49: L14.

Date. (1929)	Size of fish. Lth.	Size of fish. Wt.	Wt./Lth.³	Growth per period. Lth.	Growth per period. Wt.	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Food per day during each period. (gm.)	Cumulative growth. Lth.	Cumulative growth. Wt.	Total food to date from June 24 (gm.)	Gm. of food per 1·0 gm. increase in wt. of fish to date.
July 15	23·0	90·1	0·007	—		—	—	—	—	—		—	—
,, 22	23·0	87·6	0·007	nil	− 2·5	31·7	—	7	4·5	nil	− 2·5	32	—
Aug. 5	23·1	97·3	0·008	0·1	9·7	96·7	10·0	14	6·9	0·1	7·2	128	17·8
,, 19	23·4	112·5	0·009	0·3	15·2	105·9	7·0	14	7·6	0·4	22·4	234	10·4
Sept. 2	23·6	122·6	0·009	0·2	10·1	110·7	11·0	14	7·9	0·6	32·5	345	10·6
,, 16	23·8	135·7	0·010	0·2	13·1	113·3	8·6	14	8·1	0·8	45·6	458	10·0
,, 30	24·3	151·2	0·011	0·5	15·5	115·2	7·4	14	8·2	1·3	61·1	573	9·4
Oct. 14	24·7	165·7	0·011	0·4	14·5	114·4	7·9	14	8·2	1·7	75·6	687	9·1
,, 28	25·3	174·5	0·011	0·6	8·8	94·3	10·7	14	6·7	2·3	84·4	782	9·3
Nov. 11	25·6	186·5	0·011	0·3	12·0	85·5	7·1	14	6·1	2·6	96·4	867	9·0
,, 25	25·7	184·8	0·011	0·1	− 1·7	37·7	—	14	2·7	2·7	94·7	905	9·6
Dec. 9	25·7	179·2	0·011	nil	− 5·6	7·8	—	14	0·6	2·7	89·1	913	10·3
,, 23	25·7	179·1	0·011	nil	− 0·1	12·5	—	14	0·9	2·7	89·0	926	10·4

TABLE 50.

Showing Percentage Growth in Weight of Specified Male and Female Plaice for each Period at Cawsand and Lympstone.

	[A] *Cawsand.*				[B] *Lympstone.*			
Date [1929]	B1 ♀	B4 ♀	D3 ♂		Date [1929]	L15 ♀	L16 ♀	L11 ♂
May 22	—	—	—					
June 6	29·2	40·5	—					
20	12·9	23·1	16·1		June 24	—	—	—
July 5	14·3	14·1	25·0		July 8	19·4	21·0	—
19	17·5	17·8	33·3		22	30·0	15·4	26·2
Aug. 2	13·8	17·4	23·3		Aug. 5	16·2	18·3	9·0
16	14·0	19·8	12·2		19	25·6	19·8	24 6
30	13·1	9·1	24·1		Sept. 2	18·4	17·6	16·8
Sept. 16	15·2	15·1	27·2		16	7·4	11·1	9·2
Oct. 1	12·6	9·9	20·6		30	22·5	12·7	15·8
15	7·3	7·8	13·9		Oct. 14	10·0	15·0	12·4
29	2·6	13·3	6·1		28	11·1	6·2	8·1
Nov. 13	5·6	5·9	7·9					
27	1·4	—	1·9					

TABLE 51.

MAINTENANCE OF MALE PLAICE AT CAWSAND (1928 AND 1929) AND AT LYMPSTONE (1929). SUMMARISED DATA.

Exp.	Fish No.	Average length (cm.).	Average weight (gm.).	Average daily ration (gm.).	Food per day/ /average body-wt.	Tables for reference.	Period to date given below.
C. 1928	Mean A	16·2	42·3	0·7	0·017	1	Nov. 30
C. 1929	Mean C	12·8	17·6	0·4	0·024	8	July 5
,,	C5a	14·6	30·0	0·6	0·019	9 & 12	Nov. 28
,,	C4a	15·9	39·0	0·6	0·015	10 & 12	Nov. 13
,,	C6a	16·7	43·0	0·7	0·016	11 & 12	Nov. 28
L. 1929	L1	17·3	52·2	0·9	0·017	28 & 33	Nov. 25
,,	L4b	20·9	76·0	1·1	0·015	29 & 33	,,
,,	L3	21·7	107·3	1·3	0·012	30 & 33	,,
,,	L2	22·9	129·7	1·4	0·011	31 & 33	,,

TABLE 52.

MAINTENANCE OF FEMALE PLAICE AT CAWSAND (1928 AND 1929) AND AT LYMPSTONE (1929). SUMMARISED DATA.

Exp.	Fish No.	Average length (cm.).	Average weight (gm.).	Average Daily ration (gm.).	Food per day/ /average body-wt.	Tables for reference.	Period to date given below.
C. 1928	Mean B	14·2	29·6	0·6	0·021	2	Nov. 30
,,	Suggested mean B	—	25	0·4 to 0·5	0·016 to 0·020	—	,,
C. 1929	Mean A	13·7	22·3	0·4	0·019	8	July 5
,,	A4a	16·3	40·5	0·6	0·016	13 & 16	Nov. 27
,,	A6a	16·3	42·0	0·6	0·015	14 & 16	,,
,,	A3a	17·7	56·5	0·8	0·015	15 & 16	,,
L. 1929	L5	18·5	64·8	0·9	0·014	34 & 38	Nov. 25
,,	L6	19·2	70·6	1·2	0·017	35 & 38	,,
,,	L7	22·5	102·7	1·3	0·013	36 & 38	,,
,,	L8a	22·0	103·2	1·3	0·013	37 & 38	Sept. 30
,,	L8b	23·1	113·8	1·4	0·013	37 & 38	Nov. 25

TABLE 53.

Maximum Requirements and Growth of Cawsand and Lympstone Male Plaice.

Exp.	Fish No.	Initial size.		Final size.		Growth.		Total No. of days.	Total food taken. (gm.)	Average weight of food per day. (gm.)	Food per 1·0 gm. increase in weight of fish. (gm.)
	Mean	Lth.	Wt.	Lth.	Wt.	Lth.	Wt.				
C. 1928	C	16·0	38·8	22·6	123·3	6·6	84·5	176	1129	6·4	13·3
C. 1929	D3	12·0	15·5	21·0	105·0	9·0	89·5	175	585	3·3	6·5
,,	D2	12·2	15·0	17·9	64·0	5·7	49·0	175	471	2·7	9·6
,,	D4	12·0	13·0	17·3	56·5	5·3	43·5	175	475	2·7	11·7
,,	D1	12·2	14·0	15·9	40·5	3·7	26·5	160	420	2·6	15·8
,,	D5	11·9	14·5	15·8	38·0	3·9	23·5	190	424	2·2	18·0
L. 1929	L11	16·1	23·2	23·1	157·4	7·0	134·2	154	953	6·2	7·1
,,	L12	18·1	44·0	21·8	111·4	3·7	67·4	154	758	4·9	11·2
,,	L10	16·6	36·2	19·1	82·8	2·5	46·6	98	536	5·5	11·5
,,	L9	19·7	57·5	21·3	102·6	1·6	45·1	98	676	6·9	15·0

TABLE 54.

Maximum Requirements and Growth of Cawsand and Lympstone Female Plaice.

Exp.	Fish No.	Initial size.		Final size.		Growth.		Total No. of days.	Total food taken. (gm.)	Average weight of food per day. (gm.)	Food per 1·0 gm. increase in weight of fish. (gm.)
	Mean	Lth.	Wt.	Lth.	Wt.	Lth.	Wt.				
C. 1928	D	16·1	43·3	23·3	151·2	7·2	107·9	176	1129	6·4	10·5
C. 1929	B4	13·2	18·5	21·3	103·5	8·1	85·0	189	613	3·3	7·2
,,	B1	13·2	24·0	21·0	105·5	7·8	81·5	189	618	3·3	7·6
,,	B5a	17·9	54·0	20·1	86·0	2·2	32·0	71	291	4·1	9·1
,,	B6	12·0	16·0	16·7	51·0	4·7	35·0	189	448	2·4	12·8
L. 1929	L16	18·4	50·5	24·1	175·0	5·7	124·5	154	1060	6·9	8·5
,,	L15	15·1	31·9	23·0	140·7	7·9	108·8	154	883	5·7	8·1
,,	L14	23·0	90·1	25·7	184·8	2·7	94·7	133	907	6·8	9·6
,,	L13	15·1	27·0	19·2	70·4	4·1	43·4	154	626	4·1	14·4

The Larvæ of the Plymouth Galatheidæ. I. *Munida banffica, Galathea strigosa* and *Galathea dispersa.*

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With 1 Text-Figure and Plates 1–3.

In the Plymouth district there are five or six species belonging to the Galatheidæ, one Munida, and four or five Galathea. The species occurring are the following :—

Munida banffica (Pennant).
Galathea strigosa L.
Galathea dispersa Kinahan.
Galathea nexa Embledon.
Galathea squamifera Leach.
Galathea intermedia Lilljeborg.

Of the Galathea species, it is a disputed point as to whether *Galathea dispersa* and *G. nexa* are separate species. Both occur in our outside waters, the *dispersa* form being exceedingly common, the *nexa* form rare. Selbie (1914), who discusses the question in detail, regards the *nexa* form, which is more spiny and has shorter claws, merely as an old male of *dispersa*, giving them both the earlier name of *nexa*. Crawshay (1912) found both forms occurring on the outer grounds beyond the Eddystone and states that the males and females of *nexa* both had these characteristics and were easily distinguished from *dispersa*. Unfortunately I have been able only to examine one *nexa* but many *dispersa*, and the *nexa* form was quite different in appearance. It was a small male, probably not fully grown, but its claws were much shorter and more spiny than the males of *dispersa* of the same size. No live specimens of *nexa* have been available recently and therefore no berried females from which to obtain larvæ. The larvæ of *dispersa* have been hatched from the egg and it has been seen clearly that these are by far the commonest Galathea larvæ in the plankton of the outside waters and occasionally inshore. The larvæ of *Galathea strigosa* are frequently found with those of *dispersa* outside and also occur inside with those of

G. squamifera, which is almost exclusively an inshore form. Those of *G. intermedia* occur occasionally both outside and inside.

These four larvæ are easily distinguishable and are the only Galatheas to be found after careful search in the outside plankton, usually taken beyond the Eddystone. It does not follow, however, that there may not be another larva occurring still farther out, which may belong to *nexa*. It is regarded here as probable that the two species are distinct and therefore the species called *nexa* by Selbie (*loc. cit.*), and also by G. O. Sars (1890) when describing the larva, is here called *dispersa*, and it is presumed that the five species of Galathea occur in the district. Of these the larva of *G. nexa* is not yet known. *Munida banffica*, only recognised recently as present in the Plymouth fauna, but now found to be abundant, has been followed through all its larval stages and hatched from the egg. The larvæ agree in all respects with those of Sars (*loc. cit.*) who described and figured them as *Munida rugosa*. *Galathea strigosa* and *Galathea dispersa* have also been followed through the whole of their life-histories and hatched from the egg, *G. strigosa* for the first time, and *G. dispersa* agreeing with Sars' description of that species, described as *G. nexa*. *Galathea squamifera*, the common shore species, has not yet been hatched from the egg, but has been followed through all its larval stages until the young Galathea emerged from the last larva. *Galathea intermedia*, not nearly so common, has been found in all the larval stages, except the second, and has been kept until the last larva turned into the young Galathea. The last two species have also been described by Sars (*loc. cit.*) to a certain extent. They are reserved for a later paper in the hopes of hatching them from the egg. It is possible to recognise all these four larvæ, particularly when alive, as the colouring in each is characteristic, but they can also be recognised when preserved, by other characters as will be shown in the following notes :—

The general form of the larval galatheid is well known, Sars having described the general life-history of Munida and of Galathea with details of the appendages. Munida is easy to distinguish from Galathea, but the species of Galathea have hitherto been difficult to separate from one another. In an attempt to differentiate all species of the decapod larvæ from Plymouth it has been possible to distinguish these four distinct Galathea larvæ.

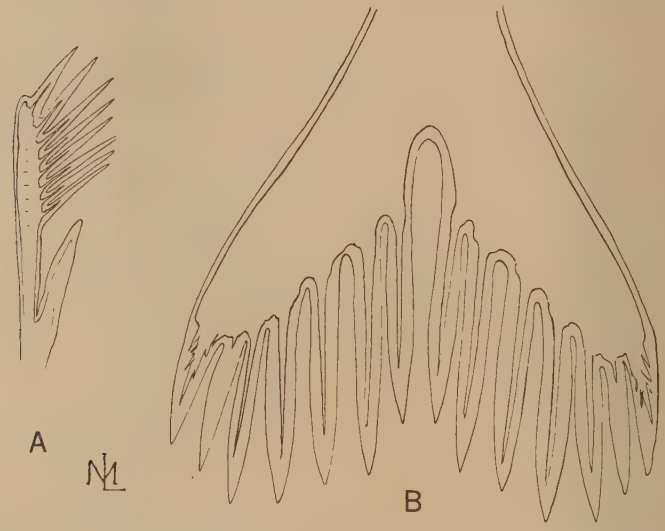
Munida banffica, *Galathea strigosa* and *Galathea dispersa* all occur fairly commonly on the outside grounds, *G. dispersa* being much the most numerous. *G. strigosa* and occasionally *G. dispersa* also occur inshore, but Munida is always outside. All occur in berry at least from autumn to spring and probably (certainly in the case of *G. dispersa*) all the year round, and the larvæ are fairly abundant in the plankton, particularly the two Galathea species.

Munida can be distinguished at once from Galathea by its elongated form, long spines and aciculate antennal scales. *Galathea strigosa* can be distinguished from the other Galathea larvæ by its longer spines and larger size, except in the last stage of *G. dispersa*, which is much the same size but easily separated by having spines on the fifth abdominal segment and not on the fourth. *G. squamifera* and *G. intermedia* are much smaller and although *squamifera* has spines on the fourth abdominal segments, they are much shorter than in *strigosa* and the other spines altogether smaller. *G. intermedia*, having spines on the fifth abdominal segment only, is so much smaller than any of the others that there is no mistaking it. All four can be separated when alive by their colour.

The common features of Munida and Galathea larvæ are the following : four or five larval stages ; very transparent body with orange-red pigment in certain areas, rarely a little brown, yellow or crimson ; long rostrum ; carapace produced into a spine posteriorly at each side, the margin of the carapace toothed behind laterally and on the hind margin ; eyes very conspicuous ; antennules with long base ending in two short branches, the inner with a long seta at its tip, which later disappears, and one on the inner side of the base takes its place, the outer with æsthetes and spines ; antenna with inner flagellum unjointed in all stages, the scale ending in a long apical spine and bearing several setæ internally, mandible with a simple palp in later stages ; first maxilla with two inner lobes and either a one- or two-jointed palp ; second maxilla with four inner lobes, an unjointed palp, and a conspicuous external plate. Only two pairs of maxillipedes functional in the first larva with swimming exopodites and jointed endopodites, the third pair rudimentary and only functional in the second stage. Abdominal segments denticulate, or with two spines dorsally on the hind margins, with lateral spines on the fourth and fifth segments, or on the fifth only. Telson deeply forked, armed at the end with seven spines on each side in the first stage, eight in the second ; the second spine from the outside being hair-like, the fourth becoming the longest. In the first and second stage the telson is a simple plate fused with the sixth segment, and with the outer spine the longest ; in the third, fourth and fifth (if present) uropods appear, and the telson is cut off from the sixth segment. In the third stage the telson is long and narrow, uropods are formed but their base is not cut off and there are no setæ on the inner branch, the outer branch bearing several setæ. In the fourth stage (usually the last) the inner branch is setose and the base is cut off from the uropods. The legs appear gradually until at the last stage the first is large and chelate and bulges out from the body, but no legs are functional. Pleopods appear as small knobs in the third stage and are long but unjointed in the last stage. Four larval

stages appear to be the usual number, but in *Galathea dispersa* there are four or five, five being the most frequent. By keeping the third and fourth larvæ of all the species it was found that the young Galathea emerged from the fourth larva in Munida, *Galathea strigosa*, *G. squamifera*, and *G. intermedia*, but although *G. dispersa* sometimes emerged as a young Galathea from the fourth stage, there was in this species a fifth stage which seemed to be usual. The fourth larva was seen emerging from the third in all the species.

The embryonic cuticle is cast off just before hatching. It corresponds to the pre-zoea of the Brachyura. It is very interesting, for in the telson



Text-Fig. 1.—*Munida banffica*. A, embryonic antenna; B, embryonic telson.

seven fully developed long spines cover the seven setæ of the first larva (Text-Fig. 1B), the second, which in the larva is represented by a hair, having quite a normal large spine covering it. The antennal scale is covered by a sheath with eight large spines on the inside corresponding to the eight setæ in the first larva (Text-Fig. 1A).

Sars (*loc. cit.*) has described and figured the appendages very fully both in Munida and Galathea; it is therefore unnecessary to figure them again, but the chief differences and the general aspect of the larvæ are given below. The red colouring of the first larva is indicated in the figures of the plates by black. The same scheme occurs through all the larvæ of each species, but intensified in the later stages.

Genus Munida.

Munida banffica (Pennant).

(Plate 1).

Larvæ described by Sars (1890) as *M. rugosa*. Four larval stages (no fifth stage seen), the fourth changing to a young Munida. First larva from egg, second larva from first from plankton, third from second from plankton, fourth from third from plankton, first young stage from fourth from plankton.

Eggs 0·80 mm. by 0·64 mm. when nearly ready to hatch; at first orange-red, then becoming brown.

Larvæ very transparent, orange-red dorsally under the carapace in two distinct streaks and a small patch each side, two streaks on each side of the abdominal segments and at the base of the telson. Length of first larva from tip of rostrum to end of telson 6·24 mm. (Plate 1, Figs. A and B), growing rapidly so that each successive stage is conspicuously larger than the last. The size varies to some extent and a third larva of one brood may be smaller than a second larva of another brood. Rostrum very long, prickly, base of antennule long, antennal scale aciculate with long drawn-out prickly spine at the tip, carapace usually covering the first segment and sometimes part of the second, with long lateral posterior spines. Second to fifth abdominal segments with two dorsal spines on the hind margin, dwindling in size in the later stages; conspicuous lateral spines on segments four and five. In the third and fourth stages a large central spine on the hind margin of the sixth segment. Outer branch of uropods with a long apical prickly spine. Outer spine of telson very long and armed with thick spines in the early stages, dwindling later; second spine hair-like, fourth becoming the longest. In the third stage there are five smaller spines on each side internal to the fourth, and in the fourth stage there are six, making ten in all on each side; unlike Galathea which has only eight on each side. Palp of second maxilla one-jointed (two-jointed in Galathea). Gills conspicuous in last stage.

Genus Galathea.

All the larvæ of Galathea differ from Munida in being much shorter with non-aciculate antennal scales, although the scale ends in a long point, the rostrum and spines of carapace not so long. The second to the fifth abdominal segments are armed on the posterior margin with a row of minute denticles. No central spine on sixth segment. Telson with outer spine in early stages not so long, in later stages with eight spines on each side. Second maxilla with two-jointed palp. Otherwise much like Munida.

Galathea strigosa L.

(Plate 2).

Four larval stages (no fifth seen), the fourth changing to a young Galathea. First larva from egg, second from first from plankton, third from second from plankton, fourth from third from plankton, young Galathea from fourth from plankton.

Eggs 0·88 mm. by 0·76 mm. when ready to hatch. At first orange-red, then brownish.

Larvæ very transparent but with more colour than Munida. Orange-red on base of antennules on side of eyes, on exopodite of maxillipedes, at base of rostrum and a large mass in the thoracic region. Two large streaks on the abdomen from the fourth segment to the base of telson. Tip of rostrum and carapace spines becoming pinkish in later stages, not prickly. Length of first larva *ca.* 3·5 mm., second *ca.* 4·96 mm., third *ca.* 5·36 mm., fourth (last) *ca.* 6·88 mm. Much less elongated than Munida, rostrum and spines of carapace and antennular scale not so long. Fourth and fifth abdominal segments with conspicuous lateral spines. Hind margin of segments two to five denticulate, the denticles dwindling and almost disappearing in the later stages. Telson a wide triangular plate in the first and second stages, having seven spines on each side posteriorly in the first stage, eight in the other stages ; second spine hair-like. In the later stages the telson gradually narrows and the fourth spine becomes much the longest. Nine or ten spines in the inner uropods on the fourth (last) stage. This stage emerges from the third stage with no spines on the inner uropods.

Galathea dispersa Kinahan.

(Plate 3).

Larva described by Sars (1890) as *G. nexa*. It differs from *G. strigosa* in its smaller early stages, absence of lateral spines on the fourth abdominal segment, narrower telson, and in its pigmentation.

Four or five larval stages. Five is probably normal, but the young Galathea has been seen to emerge from the fourth stage, and the normally fifth stage has been seen to emerge from the third stage with no spines on the inner branch of the uropods ; thus the fourth or fifth stage may be omitted.

Eggs measure 0·64 mm. by 0·48 mm. when nearly ready to hatch. At first orange-red, then brownish.

Larvæ very transparent, first obtained from egg, second from first from plankton, third from second from plankton, fourth from third from

plankton, fifth from fourth or occasionally from third from plankton, young Galathea from fourth or normally from fifth from plankton.

Orange-red on base of antennules, on maxillipedes, at base of rostrum, much on thorax, on first, second and third abdominal segments (not always on third), on the fifth and at the base of the telson in two streaks. In the later stages there is a good deal of yellow in the thorax. Rostrum and carapace spines becoming pinkish, smooth. First larva *ca.* 2·5 mm., second *ca.* 3·1 mm., third *ca.* 3·9 mm., fourth *ca.* 5 mm., fifth *ca.* 6·56–6·8 mm.

The young stages of Galathea from the last larva are much alike, but Munida is easily recognised by its three-pronged rostrum (Plate 1, Fig. F), the long central spine having a small tooth each side near the tip. The first young stage of *Galathea strigosa* and *G. dispersa* closely resemble the figure of *G. intermedia* given by Sars. All have a fairly long pointed rostrum with three prominent teeth on each side and usually with one or two smaller teeth at the base, and five prominent teeth at the sides of the carapace, usually with smaller teeth in between. The legs bear many spines and hairs but external specific characters are hardly apparent. *Galathea dispersa* (Plate 3, Fig. J) has a longer rostrum and more prominent teeth on the carapace than *G. strigosa*. Both are of a yellowish colour with red, no blue being apparent as yet in *G. strigosa*.

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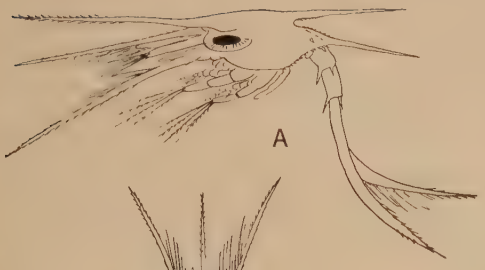
EXPLANATION OF PLATES.

(All drawn to same scale.)

PLATE 1.

Munida banffica.

A, B. 1st larva from egg, 6·24 mm. long.
C. 2nd larva from first from plankton.
D. Posterior end of 3rd larva from plankton.
E. Posterior end of 4th larva from plankton.
F. Carapace of 1st juv. from last larva from plankton.



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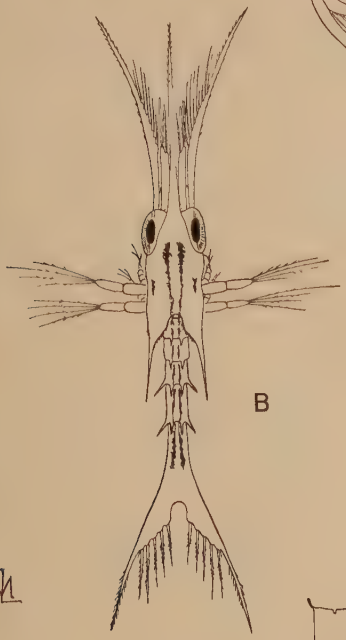
F



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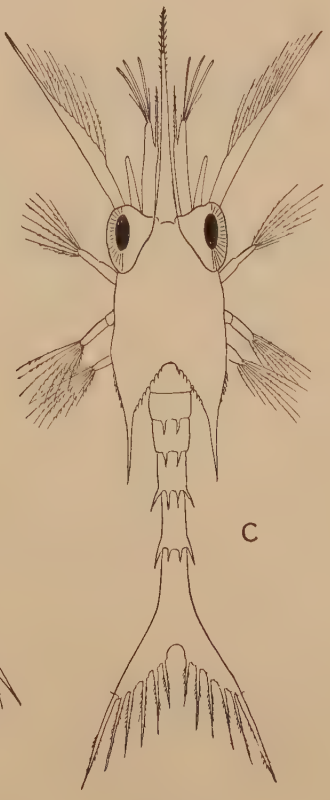

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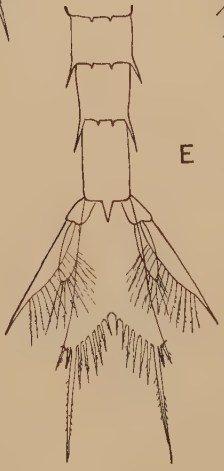

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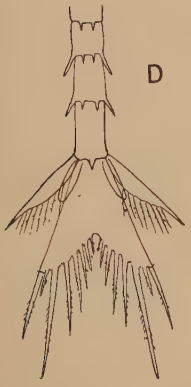

D

PLATE 2.

Galathea strigosa.

A, B. 1st larva from egg, 3·5 mm. long.
C. 2nd larva from plankton, 4·96 mm. long.
D. 3rd larva from plankton, 5·36 mm. long.
E. 4th larva from plankton, 6·88 mm. long.
F. Carapace of 1st juv. from last larva from plankton.

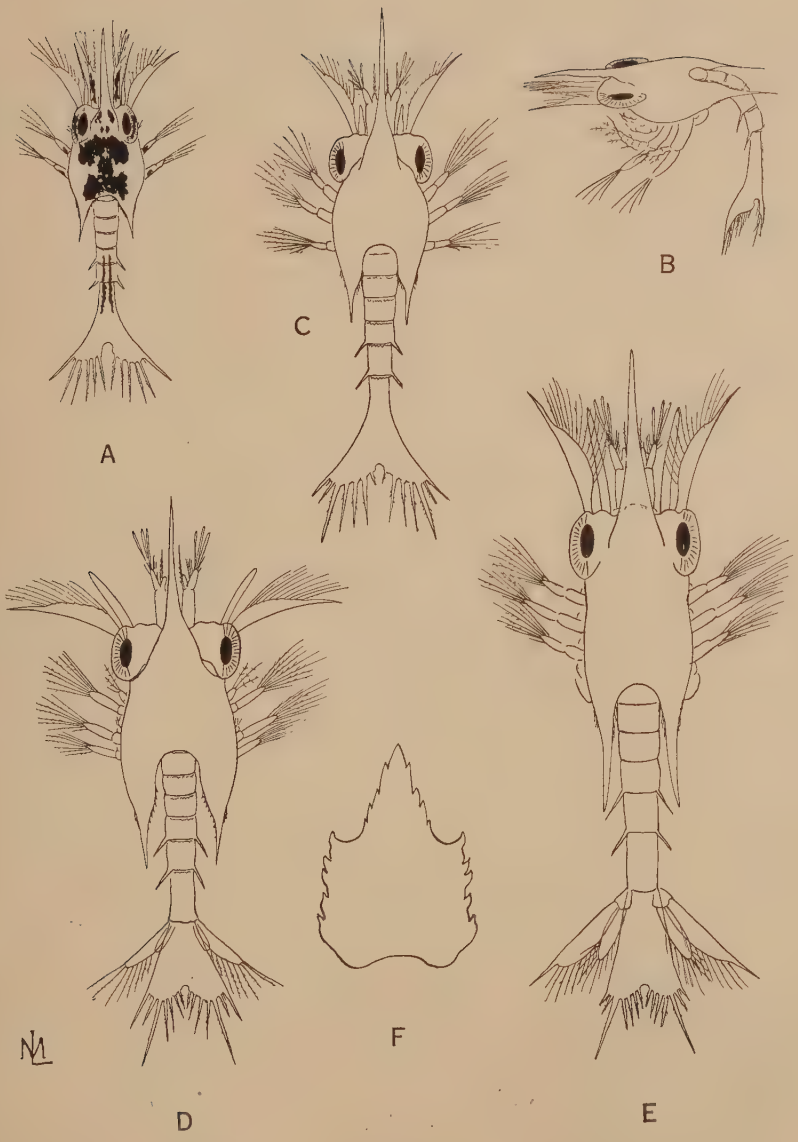
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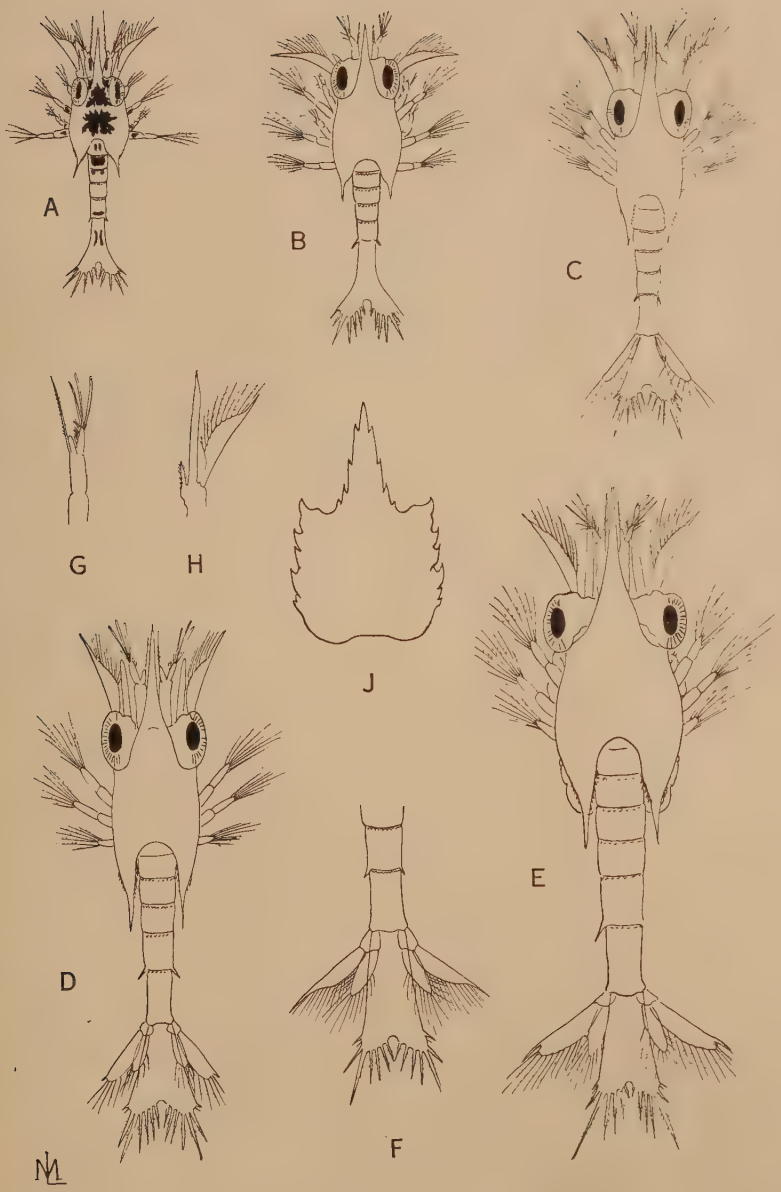
PLATE 3.

Galathea dispersa.

A. 1st larva from egg, 2·5 mm. long.
B. 2nd larva from plankton, 3·12 mm. long.
C. 3rd larva from plankton, 3·9 mm. long.
D. 4th larva from third, 5 mm. long.
E. 5th larva from fourth, 6·56 mm. long.
F. Posterior end of a larger fifth larva.
G. Antennule of same.
H. Antenna of same.
J. Carapace of 1st juv. from last larva.


A
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E

Some New Eye-Colour Changes in *Gammarus chevreuxi* Sexton. Part I.

By

E. W. Sexton, A. R. Clark and G. M. Spooner.

With Plate VIII.

PAGE

1. Introduction 189
   - Summary of Mutant Stocks giving Red Eyes 189
   - The Types of Eye-colour 193

2. History of the Experiment 194

3. Eye-colours and Colour Changes in Mutant Stock V 198
   - The appearance of Red Eyes in the Stock 198
   - The appearance of Purple Eyes in the Stock 202
   - Changes of Eye-colour in individual animals 203

4. Interpretation of Colours in Terms of Pigmentation 204
   - Darkening and Lightening 204
   - The Interpretation of Purple 205
   - Relation between the various colours. 206

5. Results of Matings within Mutant Stock V 206
   - General 206
   - Charts 1–4 207
   - Table II 215
   - Value of Data for Genetical Interpretation 215

6. Summary 217

1. INTRODUCTION.

Summary of Mutant Stocks giving Red Eyes.

The eye-colour of the wild *Gammarus chevreuxi* is always black and no variation from the normal Black has ever been observed in the many thousands which have been taken in the native habitat of the species. This, however, does not preclude the possibility that mutations may have arisen in the wild stock and have gone under in the struggle for survival.

The difficulty we have had throughout our work in establishing mutant stocks that have arisen in the Laboratory indicates that the mutations of the type used for genetical study would have but little chance under

normal conditions in nature, of survival through the early critical period. Each new mutation has shown greatly lowered vitality during its earlier generations, accompanied by marked abnormalities in breeding.

But once established, the mutants tend to become healthier with each generation, some, such as the Red-eye and the Albino-eye of Mutant Stock I, for example, being able, in time, to hold their own with the normal wild Black-eyed animals in vigour, length of life and number of offspring.

As has been shown before (**5**, p. 46), the black pigment in normal cases starts as bright scarlet in the embryo and passes through various darkening stages from "intermediate red" to "dark red" and then to jet black on hatching.

In all the different mutating stocks there have arisen Red-eyed specimens in which the red colour that normally appears only for a short time in the embryonic stage persists after hatching. It is noteworthy that the Reds from the five stocks with which we work are all genetically distinct from one another, for in whatever way we cross-mated the Reds from different stocks the young have always been born Black-eyed. In other stocks started at the same time as Stock V, in which Red-eyes appeared, none survived to be cross-mated; the details of their occurrence are given below (p. 00).

The term "Mutant Stock" we apply to the offspring of any one particular Black-eyed pair in which mutations from the normal have developed. They may be summarised briefly.

Mutant Stock I.

One of the pairs brought in from the wild on June 12th, 1912, and kept in laboratory conditions, gave the first Red-eyed young in the F_2 generation from one Black-eyed female only—the Red a bright scarlet (Plate VIII Normal Red). Other mutations in this stock were, the Albino degenerate eye from a mating of Black with Red, the Irregular Coloured eye, the No-white, the Blind, and the One-eyed. This is the stock with which Mr. E. B. Ford is working at Oxford.

Mutant Stocks II, III, and IV.

These three stocks came from a dredging brought in on September 12th, 1922. Nineteen pairs were set out in an incubator kept as nearly as possible at 21° C. Only two of the pairs gave redness in their offspring (Stocks II and IV), both in the F_2 generation.

Sixteen other pairs were started in laboratory conditions, with the temperature varying through a wide range, from two to six degrees centigrade in the twenty-four hours; the highest recorded was 27° C. in July, the lowest 5·7° C. one day in January, when the heating apparatus

failed. Five of these pairs, one the Mutant Stock III referred to below, gave some redness in the offspring. Of the others, one showed slight reddening in F_1 and F_2, two in F_1 only, and the fourth gave two bright Reds out of a total of 574 offspring.

Mutant Stock II. The Red-eye* in this, as in Stock I, is bright scarlet associated, as in all the stocks, with normal pale-green body-colour. The most striking feature appeared with the Red-eye, in the F_2 generation, viz., the White-body mutation, in which the body and eye are completely devoid of coloured pigment. The eyes connected with it are, the homozygous White-eye, the Changeling White which later develops red-eye and body colour, the Flushed White, and the Purple White eye.

Mutant Stock III differs from the others in that the Red came gradually, some Black-eyed parents becoming Reddish Black and giving different shades of Red-eye in their offspring. This Red is not the bright scarlet of Stock I but a deeper tint, like the New Red figured on Plate VIII. Another point of resemblance with our new mutation, V, is that the eye-colours sometimes change with growth, but with these it is usually only in the one direction, a *lightening* of the colour, e.g., Reddish Black to Dark Red, Dark Red to brighter Red.

The "Mosaic" eye appeared in this stock, i.e. an eye with some of the ommatidia jet-black and some bright red.

In *Mutant Stock IV* also there are different kinds of Red eyes, bright, intermediate, and dark. Like the preceding, the Dark Red lightens in varying degree. The colours are heritable, and an animal hatched Dark Red functions always as Dark Red, however much the colour may alter during its life.

In our new *Mutant Stock V* with which this paper deals there is much greater variation in eye-colour and, particularly, in colour-changes. All intermediate stages between Black and Red have occurred. Changes undergone after birth include both darkening and lightening, as well as either permanent or temporary changes to some shade of purple. Intermediate Reds have been crossed with Reds of Stock I, Stock II, and Stock III and have given a Black F_1 with all.

In comparing these five mutant stocks, it is seen that there are two distinct categories of mutants producing red eye-colour. In one, represented by Stocks I and II, the Red-eye appears as a typical mutation, arising suddenly and persisting as a simple recessive. Its mode of inheritance is comparable with that of other familiar recessives, occurring, for example, in Drosophila or Primula, and interpretable in terms of a change

* This Red is referred to as "New Red" in "Inheritance in *Gammarus chevreuxi* Sexton" (**3**, p. 119). Since then, we have found that the different Reds of the five stocks fall into two distinct classes of colour—the bright scarlet of Stocks I and II, which we call "Normal Red," and a deeper, more intense shade of red which appears in Stocks III, IV, and V, to which we now confine the term "New Red" (see Plate VIII).

in a single gene. Attention may be called, firstly, to the manner in which the Red-eye of Stock I originated : there were altogether 9 reds in the progeny of a certain black-eyed F_1 female,—2 out of 13 survivors from two broods with a brother, 4 out of 44 from a mating with a freshly captured male, and 3 out of 39 from another mating with the same brother. Since neither of these males threw any red offspring when mated with other females, it suggests that the female parent was in some way responsible for the occurrence of the red mutation in the offspring (**1**, p. 22). Secondly, to the physiological difference between " Black-eye " and " Red-eye " as demonstrated by Ford and Huxley (**4**, p. 115), who showed that when kept at a temperature of 23° C. the red-eyed individuals gradually darken to black, undergoing a process similar to, but much slower than that of normal blacks before hatching. The Red-eye is thus a case in which the rate of deposition of black pigment is very much reduced, in fact indefinitely reduced at normal temperatures. A more precise idea of the action of the gene concerned in the Red mutation is thus obtained. The mutation " Red-eye " is now expressed as a certain degree of retardation of pigment deposition.

In the other category, represented by Stocks III and V, redness emerges somewhat gradually, as described below. Intermediate stages between black and red occur and it seems as though segregation is necessary for the production of a bright red eye. Colour change during the course of life, involving either darkening or lightening, is of usual occurrence and characteristic of these stocks.

The manner in which red eye-colour appears is as follows. A certain number among the offspring from a wild pair are found to develop a reddish tinge and may become " Reddish Black " or even redder. Amongst the next generation, whether derived from these specimens or from unchanged Blacks, there occur individuals born in various stages of redness, i.e. " Reddish Black," " Dark Red," " Intermediate Red " (for explanation of these colours see below, p. 193 and Plate VIII), and bright " Reds " may also appear. Those that survive of the various reds may keep the same colour throughout life, but they are more liable to change, either in the direction of red (lighten) or black (darken). In Stock III lightening was the rule, but in Stock V both darkening and lightening occur. One other point common to the Stocks III and V should be mentioned—namely, that the shade of the red colour in the " New Red " eye is different from that in the original " Normal Red " (see Plate VIII). Examination of specimens in Stock V indicates that this is because the New Red is rendered slightly impure by a dilute deposit of a dark pigment, apparently some form of melanin.

Stock IV holds an interesting intermediate position. The red is

evidently a simple recessive to black, but the red may exist in different shades, i.e. bright Red, Intermediate Red, Dark Red, or even Reddish Black. In so far as this stock has been investigated no tendency for the reds to darken has been found,—on the other hand nearly all the darker shades have lightened to red. Among the blacks, there are some which develop a certain degree of redness. It may be supposed that these are heterozygotes showing not quite complete dominance, but, if so, not all heterozygous specimens behave thus. The quality of the red colour is more akin to that of the " New Red " than to that of the " Normal Red."

It is hoped to enter more fully into the comparison of the various red mutants on a future occasion. The present investigation aims at elucidating the mode of inheritance of this second category of eye redness. Owing to its manner of origin there are obvious difficulties in thinking of the " New Red " eye as a simple mutation involving only one gene, however much the action of the latter may be modified by accessory genes. If, on the other hand, a group of genes is involved, then the " New Red " is not a simple, but a multiple recessive. The comparison, therefore, of the " New Red " with the " Normal Red " becomes a matter of considerable interest, and may well throw light on the general problem of the nature of mutations.

The Types of Eye-colour.

We may now summarise the various shades of eye-colour that have been distinguished and used in description.

The symbols relating to each are inserted below in brackets, and the colours are shown on Plate VIII.

Pure Black (B), the normal wild type ;
Reddish Black (RB) ;
Dark Red (DR) ;
Intermediate Red (Int. in Charts 1–4, I.R. in Table II) ;
Bright Red (R), the " New Red " of this new Mutant Stock V ; the bright Red of Stock I is shown on the Plate as " Normal Red " ;
Purplish Black (PB) ;
Dark Purple (P) ;
and Reddish Purple (Rp).

Red, Intermediate Red, Dark Red, Reddish Black and Black form a series of progressive darkening, such as, for instance, occurs in the embryo of normal Blacks. Purplish Black has roughly the same depth of colour as Reddish Black, and Reddish Purple that of Intermediate Red.

Mr. E. B. Ford, in his work on *Gammarus chevreuxi* at Oxford, has distinguished a series of fourteen stages of darkening in the red eye-colour (**4**, p. 114). A proof of our Plate VIII was sent to him, and he has very kindly compared the eye-colours on the Plate with those in his series.

He allows us to state that his "stage 3, Light Red" is the same as our Intermediate Red, his "stage 6, Brown" is the same as our Dark Red, and his "Chocolate, stage 9" as our Reddish Black.

The selection of these particular colours is purely a matter of convenience. Every gradation, both among the reds and between the reds and purples, has been observed. The standards chosen are sufficiently far apart to be determinable even in a live, wriggling specimen, but they are sufficiently close to indicate any marked change in the colour of the eye.

If a "No-white" eye (i.e. lacking the reticulation of white pigment) were being used no doubt a greater accuracy in differentiation of colour shades (if indeed desirable) could be obtained. In these eyes with the normal white pigmentation, not only is the colour of the pigment cells largely hidden or shaded, but the optical effects of the white pigment when the animal is moving quite clearly give a brighter, redder shade than is seen when the eye is perfectly still. Care has to be taken especially with regard to this latter point. Previous work on eye-colour changes (Ford and Huxley, **4** and **6**) has been carried out upon No-white eyes.

2. HISTORY OF THE EXPERIMENT.

The opportunity for this investigation occurred when a number of wild pairs brought into the Laboratory in January and February, 1928, for temperature experiments, gave offspring which showed a tendency to redden, and an F_2 containing individuals of various shades of red at birth. Altogether thirty-nine pairs were set out in an incubator, thirty-two of these gave offspring and it is noteworthy that in 20 of these stocks this tendency to redness appeared. Whatever mutations are concerned therefore occurred independently in at least 20 cases. It is difficult not to conclude that some common environmental factor was instrumental in inducing them. The change over from the wild conditions in winter to those of the incubator room (starting at a temperature of 20·8° C. and rising in four months to 28° C.) is the obvious possible environmental factor.

Mutant Stock V with which this paper is mainly concerned is descended from one of these pairs.

Before giving the details of the history of the experiment, mention must be made of the many difficulties encountered in the handling of the stock, quite apart from the problems connected with the characters investigated. The broods were usually very small, numbers such as 1 to 10 being often recorded, whereas in the normal stocks the average number hatched is from 30 to 40. Then there was frequent ineffective mating and other irregularities in breeding; the proportion of young surviving to maturity

was small; but the greatest difficulty was the cannibalism of the males, which was carried on to such an extent that few of the females died a natural death.* Adults available at the right time for mating were always scarce, and therefore continual cross-matings between members of different families of the stock, often different phenotypically, were necessary in order to keep the stock going, with the consequence that progress in the direction of establishing pure lines has been slow. The mortality after eighteen months in the incubator was so great that a reduction in the temperature was necessitated. The stock has therefore been kept under the more normal laboratory conditions, which has meant that, though its viability has increased, it is no longer subject to a regulated temperature.

The incubator referred to was a small room 10 ft.×8ft.×7 ft. high, in which the temperature could be kept approximately constant. It was heated by hot-air pipes, the gas furnishing the hot air being regulated by a Hearson's thermostat placed in the room. Air and water readings were taken daily by means of a thermograph in the air, and Negretti and Zambra's maximum and minimum thermometers immersed in a covered glass dish of water, the difference between the readings for air and water averaging about half a degree centigrade, those in the water being the lower. The broods were always examined in the incubator, so that

* An instance from one of the F_4 families may be given in illustration of these points. Seventy-six young were hatched, of which only 18 survived to maturity, six males and twelve females. Only one of the females died a natural death, the other eleven were eaten by their mates.

Normally a male pairs with a female towards the end of a reproductive period, some days before her moult is due, mating as soon as this is accomplished. The eggs are laid almost at once; if not laid before the cuticle hardens, that is to say, within about 24 hours of casting the old skin, oviposition becomes impossible till the time for moulting comes again. The term "mating" therefore implies a complete reproductive period. The danger to the females comes during or just after the moult while the cuticle is still soft.

The matings of this family were as follows:—

One female mated, ate the male; mated with another male, same stock, laid eggs which were thrown off, mated again four times with no results; then ate the male; mated twice with a brother, and was eaten.

One male mated with sister female, no results, mated four times with eggs laid and broods hatched, numbering 1, 11, 14, and 21 respectively, then ate the female; mated once again with another female and died.

The second male mated with a sister, female died; mated with another sister, ate her; with a third female, had a brood of 1; mated again, brood of 8, ate the female, and died.

The third male had two matings with a sister, broods of 1 and 3, ate the female; two matings with another female, brood of 1, mated again, ate this female, and another, and then died.

The fourth male mated twice, ate the female, and died.

The fifth had three matings with a female, ate her; mated with another, ate her; mated twice with a third female, ate her; then as it had grown to a large size, it was given a female from the wild. With this one, it has had four broods, numbering 17, 46, 38, and 26 respectively, followed by three matings with no results, and now, after a fourth mating, eggs laid.

The sixth male ate two sisters in the brood bowl; mated three times with a third female, broods of 2, 9, and 8, mated again and ate the female; mated with another, ate her; mated with a female from the wild, ate her; mated with a second one, a brood of 15; mated again, brood of 22, and male was eaten.

they were not subjected to any change of temperature during the examination.

The temperature of the room when the experiment started on February 24th, 1928, was 20·2° C. The heat was gradually and steadily increased until in April it reached a mean of 25·8°* From May 1st to June 11th the mean was 26·8° C. From June 12th to June 26th it stood at 28°. This was found to be the limit of endurance for the species. Only 5 broods were hatched from all the pairs in the incubator, and these were very small numbers, 8, 3, 2, 3, 1, –all died within a few days. An alteration was therefore made, and the heat was lowered to 23·8° C. and kept at this from June 26th to July 6th. It rose again through July, as will be seen by the mean temperature given below for the incubating periods, until on August 16th it was decided to keep it constant at about 23° C. From August 16th, 1928, to June, 1929, it was kept almost constant between 22° and 23° C. In June it rose one degree and was constant through July and August till September 6th when the gas was turned out. The stock had grown so weak that it had to be re-established in healthier conditions.

The heat averaged 18° C. through the rest of September, and 15° through the winter months.

On April 9th, 1930, the bowls were brought out into the Laboratory, temperature averaging 15·7° C. The heating of the Laboratory was stopped on April 30th, and since then the mean was 14·5° for May, rising in June to about 17° C.

The first four generations, with the exception of one F_4 mating were hatched and reared in the heat. The broods hatched since are marked with the symbol † on the Charts.

Mutant Stock V is derived from one of the pairs brought in on February 13th, 1928, and kept at first in laboratory conditions for the females to extrude their young. In this pair, the female extruded her young, and laid eggs on February 16th. They were placed in the incubator on February 24th, the temperature then standing at 20·2° C. On the 28th this brood was extruded and the first batch of eggs to be laid in the heat was deposited.

Twelve Black-eyed F_1 were extruded on March 8th, the mean temperature for the incubation period, 8 days, being 20·8° C. Ten of these reached maturity about six weeks later, 6 males and 4 females, and mated in the brood bowl, but only one pair gave any results. From this pair all the stock is descended. The others mated again and again,

* At 10.30 every morning the temperature at the time was read, and also the maximum and minimum temperature for the previous 24 hours. It was found that the mean of the temperature readings at 10.30 gave a result which differed only slightly from the mean of the maximum and minimum readings. When considering the temperature over a period we have therefore used the mean of the readings taken at 10.30 a.m.

seven batches of eggs were laid and thrown off, only 1 young, Black-eyed, was hatched and it died. The pair referred to mated and were taken out in a separate bowl on April 27th, and the *first* batch of eggs was laid on April 28th.

The heat was being increased, and the mean temperature for the incubating period was 25·8° C. It was evidently too much for the female, for though she carried the eggs until they showed orange in the pouch, i.e. to within a few hours of hatching, they were thrown off.

The *second* brood, laid on May 5th, was also thrown off.

The *third* brood, laid May 12th, hatched, and 5 young were extruded on May 20th, two Black-eyed and three with a slightly reddish tinge, the first sign of redness in the stock. Four survived, eye-colour unchanged, and two, a male and female, were seen to be mature on July 6th, a period of 47 days. By July 16th both females had eggs.

The matings gave different results, viz. the Black male mated with the Reddish Black female gave 47 Black, 21 Reddish Black, 1 Dark Red, and 10 Intermediate Red; while the other pair, Reddish Black male with Black female, gave 112 Black, 37 Reddish Black, and 16 Intermediate Red.

The *fourth* brood, laid May 20th, extruded May 27th, consisted of 7 Black and 1 Reddish Black. Four survived, three males and one female, maturing in about 40 days, the first eggs, only 2 or 3 in number, being laid on July 6th. The offspring given by this female were 97 Black and 1 Reddish Black.

The *fifth* brood, laid May 27th, and the *sixth* on June 4th were both thrown off—most likely because of the rise in temperature to 26·3° C. The *seventh* brood, laid June 11th, and extruded June 18th, was also a failure, for only 2 young, Black-eyed, were produced and these died almost at once.

The temperature was still rising, the mean from June 12th to June 26th being 28° C. Two batches of eggs were laid during this period, the *eighth* on June 18th, *ninth* on June 26th, but were thrown off, and as it was evident from the mortality in all the stocks that the conditions were too severe, the gas was lowered. The next brood, *tenth*, laid July 5th and extruded on the 12th, with a mean temperature during the incubating of 23·6° C., died before the young could be examined for eye-colour.

New eggs, brood *eleven*, were laid on July 13th, extruded July 20th, the mean for the seven days being 25·3° C.; 15 Black and 1 Reddish Black were hatched, but none reached maturity.

Brood *twelve*, July 21st to July 28th, mean temperature 24·4° C., contained 13 Black, 5 Reddish Black, and 1 Intermediate Red, the first Red-eyed young of this mutation.

Five Black survived, one of which lightened to Reddish Black; five

Reddish Black also, three of them unchanged, while the other two had lightened, one to Intermediate Red, and the other first to Intermediate Red, and still further to bright Red. The one Intermediate Red of the brood, almost normal red at birth, had darkened to Dark Red by August 14th. This is the first instance of lightening and darkening both occurring in the same brood, and noteworthy too for the fact that the changes took place within a comparatively short time after extrusion, 17 days. The young were an exceptional length of time in reaching maturity, the first to be noted, a female, took 65 days to October 1st. Some of the others took 93 days, and others even longer, a period of time comparable with that taken by the young maturing in the open Laboratory.

The *thirteenth* brood, July 29th to August 6th, took eight days hatching in a mean temperature of 23·4° C. Fifteen Black and 3 Reddish Black were extruded, the only survivor, a Black female, mated, eggs thrown off, mated again and was eaten by her mate.

The *fourteenth* brood, laid on August 7th, was extruded on August 14th and 15th, mean temperature for the seven days, 23·4° C. There were 11 Black, 2 Dark Red which darkened, and 1 Intermediate Red which grew up unchanged. The first to mature took 52 days, to October 5th, the first eggs were laid on October 8th.

The male parent of these broods died on August 15th, and the female on August 16th.

3. EYE-COLOURS AND COLOUR CHANGES IN MUTANT STOCK V.

The Appearance of Red Eyes in the Stock.

From the wild pairs brought in during January and February, 1928 (see p. 194), close on 4000 offspring were hatched in the F_1 generation. All were born with black eyes, but of the 432 survivors (representing 32 families), 33 (from 14 families) showed a tendency to redden later in life. Fifteen developed a reddish tinge; fourteen became definitely Reddish Black; and four lightened still further, two to Dark Red, one to Dark Red and then to Intermediate Red, and one still further to Red.

Among the 1890 specimens in the F_2 generation, twenty-eight were born Reddish Black, two Dark Red, ten Intermediate Red, and seven bright Red. Some of these reds were born from parents that had developed a reddish tinge and some from parents that had remained black. Of the four stocks that produced these reds, namely, H. I, VIII, XXV, and XXXI (our Mutant Stock V), one, H. VIII, had shown reddening in the F_1, the other three had not.

In three stocks, H. I, XIII, and XXX in which the F_2 were all born black, subsequent reddening appeared for the first time in the F_2. Only

one of these stocks, H. I, was carried on, and in it red-at-birth specimens arrived in the F_3.

Apart from Mutant Stock V, the only survivors of all the specimens which showed any redness at birth, behaved as follows :—

One Reddish Black unchanged,

One Reddish Black became Purplish Black,

Four Reddish Blacks darkened to Black,

One Intermediate Red darkened to Black.

The figures for these stocks are given in Table I.

TABLE I.

TABLE SHOWING SIMULTANEOUS APPEARANCE OF EYE-REDNESS IN THE PROGENY OF 20 WILD PAIRS BROUGHT IN JANUARY AND FEBRUARY, 1928.

N.B.—All the pairs which gave broods are included, except 4 which gave an F_1 of under 50 and went no further, and 3 which gave still smaller F_1 with no survivors.

For explanation of the symbols see p. 193.

Stock No.	Generation.	Colour at Birth.	Survivors.
H. I	F_1 20 B		4 B un.
	F_2 102 B		10 B→**RB**.
	F_3 284B: 5**RB**: 10**Int.**		25B un.: 9 B→slightly **RB**.
	F_4 132 B: 3 slightly **RB**: 2 **Int.**		26 B un.: 4 B→**RB**: 1 B→**PB**. 1 **RB**→B: 1 **RB**→**PB**.
	F_5 26 B: 1 **RB**		4 B un.
H. VIII	F_1 127 B		26 B un.: 2 B→slightly **RB**.
	F_2 282 B: 13 **RB**: 8 **Int.**: 6 **Red**		16 B un. 1 **RB** un.: 3 **RB** →B. 1 **Int.**→B.
	F_3 221 B: 3 **RB**		22 B un.
H. XIV	F_1 55 B		26 B un.: 1 B→**RB**: 1 B→**DR** (?)
	F_2 244 B		9 B un.: 5 B→**RB**: 1 B→**RB**→B.
	F_3 62 B		11 B un.
	F_4 55 B		6 B un.
H. XII	F_1 348 B		37 B un.; 3 B→slightly **RB**.
	F_2 175 B: 2 **RB**		9 B un.
H. II	F_1 264 B		26 B un.: 4 B→**RB**.
	F_2 169 B		6 B un.: 4 B→**RB**.
H. V	F_1 369 B		32 B un.: 1 B→**RB**.
	F_2 167 B		11 B un.: 1 B→slightly **RB**.
H. XXV	F_1 234 B		19 B un.
	F_2 64 B: 1 Red		1 B un.
H. III	F_1 126 B		16 B un.: 3 B→**RB**: 1 B→**RB**→**DR**: 1 B→**DR**→**R**.
H. XLI	F_1 38 B		1 B un.: 1 B→**Int.**
H. VI	F_1 108 B		8 B un.
	F_2 9 B		4 B un.

Stock No.	Generation.	Colour at Birth.	Survivors.
H. VII	F_1 268 B		9 B un.: 2 B→slightly **RB**.
H. X	F_1 297 B		23 B un.: 3 B→**RB**.
	F_2 5 B		4 B un.
H. XI	F_1 232 B		33 B un.
	F_2 55 B		3 B un.
H. XIII	F_1 2 B		2 B un.
	F_2 73 B		9 B un.: 1 B→slightly **RB**.
H. XV	F_1 242 B		12 B un.: 1 B→**RB**.
	F_2 17 B		4 B un.
H. XX	F_1 306 B		15 B un.: 1 B→**RB**.
	F_2 69 B		3 B un.
H. XXI	F_1 141 B		8 B un.
H. XXIII	F_1 178 B		23 B un.
	F_2 65 B		9 B un.
H. XXVI	F_1 230 B		17 B un.
	F_2 41 B		8 B un.
H. XXIX	F_1 16 B		3 B→slightly **RB**.
H. XXX	F_1 23 B		3 B un.
	F_2 33 B		5 B un.: 2 B→**RB**.
H. XXXIII	F_1 33 B		8 B un.
	F_2 160 B		11 B un.: 1 B→**RB**.
H. XXXIV	F_1 43 B		4 B→slightly **RB**.
H. XL	F_1 43 B		9 B un.: 1 B→slightly **RB**.
H. XXXI	F_1 12 B		10 B un.
	F_2 65 B: 13 **RB**: 2 **DR**: 2 **Int.**		12 B un: 1 B→RB.
			7 RB un.
			1 RB→B.
			1 RB→DR.
			1 RB→Int.
			1 RB→Int.→Red.
			2 DR→RB.
			1 Int. un.
			1 Int.→DR.
	F_3 425 B: 125 **RB**: 60 **DR**: 105 **Int.**: 1 **Red**		43 B un.
			3 B→PB.
			4 B→RB.
			1 B→Rp
			1 B→Int.
			6 RB un.
			1 RB→B→PB.
			2 RB→PB
			1 RB→PB→P.
			1 RB→P.
			1 RB→DR:
			3 RB→Int.

Stock No.	Genera-tion.	Colour at Birth.	Survivors.
			1 DR un.
			7 DR→Rp.
			1 DR→Rp.→R.
			2 DR→Int.
			1 DR→Int.→Red.
			11 Int. un.
			1 Int.→RB.
			5 Int.→P.
			5 Int.→Rp.
			1 Int.→DR
			6 Int→DR→Rp.
	F_4	397 B: 252 **RB**: 233 **DR**: 131 **Int.**: 2 **Red**	113 B un.
			6 B→PB.
			9 B→RB.
			2 B→Rp.
			1 B→DR.
			2 B→Int.
			1 B→Red.
			26 RB un.
			2 RB→B→PB.
			10 RB→PB.
			1 RB→PB→P.
			1 RB→PB→Int.
			1 RB→P.
			2 RB→Rp.
			3 RB→Rp→Int.
			6 RB→DR.
			2 RB→DR→Int.
			1 RB→DR→Int.→Red.
			9 RB→Int.
			2 RB→Int.→DR.
			2 RB→Int.→Red.
			22 DR un.
			3 DR→PB.
			15 DR→RB.
			4 DR→RB→DR.
			3 DR→P.
			21 DR→Rp.
			1 DR→Int.→Rp.
			10 DR→Int.
			3 DR→Int.→Red.
			1 DR→Red.
			13 Int. un.
			1 Int.→PB.
			11 Int.→RB.
			2 Int.→P.
			1 Int.→P→DR→Rp.
			12 Int.→Rp.
			1 Int.→Rp→Int.
			2 Int.→DR→P.
			1 Int.→DR.
			1 Red→Int.
	F_5	389 B: 225 **RB**: 21 **DR**: 106 **Int.**: 18 **Red**	91 B un.
			2 B→PB.
			3 B→RB→Rp.
			1 B→DR→Int.→Red.

Stock No.	Genera-tion.	Colour at Birth.	Survivors.
			6 RB un.
			18 RB→PB→P.
			11 RB→PB→P→Rp.
			1 RB→PB→P→Rp→Int.
			1 RB→PB.
			1 RB→PB→P→Int.
			5 RB→PB→Rp.
			1 RB→PB→Red.
			1 RB→PB→DR→Int.
			2 RB→PB→Int.→Red.
			12 RB→P.
			1 RB →P→PB.
			5 RB→P→Rp.
			1 RB→P→Rp→Red.
			1 RB→P→Int.
			2 RB→P→Int.→Rp.
			1 RB→P→Red.
			2 RB→Rp→Int.→Red.
			1 RB→Int.
			1 RB→Int.→Rp.
			1 RB→DR→P→DR.
			2 RB→DR→Int.→Red.
			1 DR un.
			1 DR→RB.
			6 DR→Rp.
			2 Int. un.
			4 Int.→PB.
			1 Int.→RB.
			12 Int.→Rp.
			2 Int.→P.
			2 Int.→Rp→Int.
			2 Int.→Rp→Red.
			1 Int.→P→Rp→Red.
			3 Int.→Red.
			4 Int.→Rp→Int.→Rp.
	F_6	97 B : 12 **RB** : 266 **Int.** : 124 **Red**	10 B un.
			6 B→RB.
		Still going.	

In Mutant Stock V the 12 F_1 were all Black and remained so. One of the pairs, from which all the rest of the stock are descended, produced an F_2 containing, at birth, 65 Blacks, 13 Reddish Blacks, 2 Dark Reds, and 2 Intermediate Reds (see Chart 1). Of the 2 Intermediate Reds, one remained unchanged and one darkened to Dark Red. Of the 2 Dark Reds both darkened to Reddish Black. Of the 13 Reddish Blacks, two died young, one darkened to Black, one lightened to Dark Red, one to Intermediate Red, and one to Red, seven remained unchanged, but of these two died immature. Of the 13 Black survivors one turned Reddish Black. In this family, therefore, both lightening and darkening occurred.

The Appearance of Purple Eyes in the Stock.

The results of the matings of various members of this family with one another produced, in the F_3, an even greater variety of eye-colour changes,

which included not only darkening and lightening but also, for the first time, changes to *purple*. Purple eyes arose from various types of red and from black as well. Three shades of purple were distinguished "Purplish Black," "Dark Purple" (or simply, "Purple") and "Reddish Purple" (see Plate VIII).

In no case were young born with purple eyes, the colour in question always coming as a change later in life. Most frequently there was no further colour change after the purple condition had been reached, but this has not been the rule in subsequent generations, in which purple appears as an intermediate stage at one time or another in almost every type of colour change. It is noteworthy, however, that there is no instance at all of an eye purple at birth, the earliest onset of purpling observed up to now being at 14 days, e.g. a number of young hatched Red on April 25th, 1930, darkened to Intermediate Red by April 29th, and by May 9th were all Reddish Purple.

In the companion stocks, nearly all of which, it should be mentioned, were dying out in the F_3, purpling occurred in one family, namely, the F_4 of H. I (see Table I). One Black and one Reddish Black changed to Purplish Black.

Changes of Eye-Colour in Individual Animals.

In Stock V eye-colour changes in the individual animals have occurred in great variety. With very few exceptions they are all in the one direction—either of darkening or of lightening—that is, they do not change over from one direction to the other. Lightening is much commoner and proceeds to greater lengths than darkening, which when it occurs seldom goes beyond one stage. Purple may intervene at any point in the course of a change, as has been particularly evident since the stock was brought out of the incubator room. A glance through the accompanying charts will show the variety of occasions on which Purple eyes have appeared.

In the case of Blacks which have changed, the majority have gone no further than Reddish or Purplish Black, but there have been cases of Blacks giving Dark Red, Purple, Intermediate Red, Reddish Purple, and even Red.

Reddish Blacks have lightened to various extents, in several cases going as far as bright Red. Purple is commonly involved in the changes, especially in certain families. There are only two instances of darkening to Black.

Dark Reds have darkened to Reddish Black and in one case to Black. Of those which have lightened some have gone as far as Red.

Intermediate Reds have lightened to Red, but many more have darkened either to Dark Red or Reddish Black. As in the other types of Reds,

a number have remained unchanged, 81 changed to 27 unchanged,- curiously enough, the total proportion of changed to unchanged, taking all the Reds together, gives an actual 3 to 1 ratio, i.e. 288 changed to 96 unchanged.

Only three isolated cases of individuals born bright Red occurred in the earlier generations (F_3 and F_4). Of these two died young and the survivor darkened to Intermediate Red as it grew older. In F_5 and F_6 there have been four families which have produced a number of Reds (see Chart 1), 122 individuals altogether, but the survivors are still immature. The majority have turned Reddish Purple or Intermediate Red. Up to now, therefore, no permanent Reds, such as, e.g. those in Mutant Stocks III and IV, are known from this stock.

The Red eye of Mutant Stock V has thus occurred in two different circumstances. (1) Individuals are born red and subsequently darken or turn purple ; (2) Red occurs as the final result of a process of lightening from any of the darker reds or even black. In no case yet has the red produced by lightening undergone any further change.

4. INTERPRETATION OF COLOURS IN TERMS OF PIGMENTATION.

Darkening and Lightening.

With a view to bringing this work in line with previous work on eye-colour changes (see p. 193), it is now necessary to examine more closely the question of eye-colours and the changes described : to express them, in fact, as far as possible in terms of the pigmentation in the retinal cells. By doing this there is every chance that the problem of the colour changes will be simplified and the manner of action of whatever genes are concerned made more evident. At the least the characters will have been expressed in a less empirical form. Further, some explanation is required on the relation of the purples to other types of colour.

In the normal eye the first pigment to appear in the embryo is red. As the eye grows dark pigment is deposited in increasing quantity, the rate of deposition of red pigment rapidly falls off, and in one to two days the eye is quite black. After extrusion the rate of black pigment deposition keeps pace with the growth of the eye, so that it remains a jet-black. All the time quite probably a certain amount of red pigment is also being formed—in fact it may be a necessary precursor of the black.

The gradation of intermediate stages between the red and the black is more easily observed in cases of Normal Reds which, kept at 23° C., gradually darken during life (Ford and Huxley, **4**).

As the melanin accumulates so the shade of the eye becomes darker and the darkening is an approximate measure of the amount of melanin

accumulated. Such fluctuation as may have occurred in the concentration of the red pigment has been evidently unimportant. It should be mentioned that the extent of melanin deposition can, if necessary, be observed directly by placing the specimen in alcohol—the red pigment dissolves out, leaving behind a deposit of the insoluble dark.

In the changes occurring in Mutant Stock V and falling within the series of Black→Red, evidently a reverse process is taking place—the rate of deposition of dark pigment falls off and that of the red increases, or at least fails to decrease. Since there is reason to suppose that dark pigment, once laid down, remains always, dilution can only be caused by the growth of the ommatidia. At any given stage, therefore, there must be sufficient dark pigment to account for any earlier darker stage. Thus a specimen born Black and changing later to Reddish Black should in the latter stage contain sufficient dark pigment to account for the earlier blackness. So unless a Black starts to change early it has little chance of lightening very far. It is in fact found that few lightened Blacks pass beyond the Reddish Black stage. Again, Red individuals which have lightened from darker stages should contain a deposit of melanin. This is precisely what has been found. Several Reds have been preserved in alcohol at death, and have all shown a dilute deposit of dark pigment after the red pigment has dissolved out.

The Interpretation of Purple.

The explanation of the purple eye-colours has proved a simple matter. It was found, on examining a series of the various stages of red-eyes preserved in spirit, that the colour of the diluted dark pigment deposit which remained after the red had gone was in all cases a chocolate. The intensity of the chocolate colour, of course, varied with the quantity of pigment present, being dark for the darker red eyes and pale for the lighter red. A complete gradation, too, between dark chocolate and jet-black was found, but the quality of the colour was approximately the same all through.

Purple eyes left just the same kind of chocolate colour.

Comparison of Purple eyes in the living animals with Purple eyes preserved in spirit showed that the colour of the two was essentially the same, namely, a shade of chocolate. In fact "Purplish Black" and "Dark Purple" eyes after treatment with alcohol were found to remain unchanged; while "Reddish Purple" eyes became a duller chocolate, losing the redness. These facts go to show that the Purple colour is given by eyes in which diluted dark pigment is not covered over by red pigment. The red element in these eyes is much reduced or entirely negligible; when there is an appreciable quantity present the purple is brightened up.

Purplish Black corresponds to Reddish Black in which the red pigment

has disappeared ; Dark Purple similarly to Dark Red ; while a small admixture of red with a dilute black pigmentation gives Reddish Purple. Reddish Purple merges into Intermediate Red as the concentration of red increases.

Further examination of the effects of alcohol on different eyes has borne out this conclusion. We are indebted to Mr. E. B. Ford for the loan of spirit specimens of the Normal Red of Stock I in various stages of darkening. These specimens also showed that the dark pigment when diluted is of a chocolate colour.

To sum up, purple colour is produced by eyes in which the red pigment has fallen off without a corresponding increase in dark pigment.

The Relation between the Various Eye-Colours.

It is thus possible to express all colour variations occurring in this stock in terms of variations in the concentration of the two pigments present in the normal eye the red and the black ;* and all changes in terms of variations in the rates of deposition of each of these two pigments relative to the rate of growth of the eye (or, rather more precisely, of the ommatidia).

The relations between the various colours may be expressed by the following scheme :—

5. RESULTS OF MATINGS WITHIN MUTANT STOCK V.

General.

The matings within Stock V have at present reached the eighth filial generation. Difficulties encountered in the behaviour of the stock have already been mentioned ; and, when to these are added the complications

* As a matter of fact, in the original water-colour drawings for the colour series on Plate VIII, all the different shades were obtained by using two tones of red with black.

exhibited by the characters under investigation, it can be realised that progress towards elucidating the mode of inheritance has necessarily been slow.

For the present, therefore, we go no further than setting out the results of the various matings within the stock as they stand, in order to show the sort of results that have been obtained and the data available up to now.

Charts 1 to 4 show the cases in which in-mating within the families has been possible through to the F_8. Certain cross-matings are omitted as well as unproductive matings or matings which produced very small numbers of young. Table II, however, gives a record of every mating that has produced a brood. It is arranged so as to bring together as far as possible, irrespective of the generation, matings of a similar kind.

The known colour changes of the survivors are in all cases given. It should be pointed out that often a long period elapsed, especially in the case of the earlier generations, in between examinations of a specimen for eye-colour. Consequently in cases of colour change intervening stages have been easily overlooked. So, when a certain specimen is designated, for example, B→IR, it is not implied that there is any difference from a case denoted B→RB→DR→IR. It only means that the intervening stages that must have occurred happen to have been missed. In later generations eyes have been examined more frequently.

In each of the charts a special note is made of those families which have been reared since the stock was removed from the incubator room. It is impossible as yet to say what difference this change to a lower temperature has made. Though no doubt it may have affected the *rate* of eye-colour change, yet perhaps after all it has not made much difference to the *kind* of change. It is perhaps worth mentioning that the occurrence of purple seems much more frequent since the stock has been kept in the cool.

Chart 1.

Chart 1 shows the ancestry of the most promising of the F_6 families. Starting with the ancestral F_2 in which reddening and darkening both occur, we pass to an F_3 derived from a mating between a Black and a Reddish Black, both of which showed reddening. At birth this F_3 contained a mixture of Black, Reddish Black, and Intermediate Red of which the survivors either remained unchanged or else turned Purple. One Black changed to Intermediate Red, and there is also an indication of lightening (of the dark pigment) in the Reddish Black which turned from Purplish Black to Purple ; darkening is indicated by the five Intermediate Reds which became Dark Red before purpling, and an unusual mixture of darkening and lightening in the RB→B→PB. But it is seen that, with the exception of the one B→Int.R., there is no *reddening* such as

occurred in both the parents, and as is conspicuous in subsequent generations.

In contrast to the F_3, in the two families of the F_4 (in production of which purple parents were involved) a strong tendency to redden is shown and no change to purple appeared at all.

The three families of the F_5 are all in many ways similar. Judging from the behaviour of the high proportion of survivors, it looks as though almost every individual was doing its best to redden and turn purple at the same time. Thinking more precisely in terms of concentrations of red and black pigment, one sees that the F_5 specimens almost without exception show a steady dilution of dark pigment, while the red pigment fluctuates independently. The red pigment on the whole diminishes after birth and tends to reappear later in life, by which time the black pigment is dilute enough to produce the Intermediate Red or the bright Red condition. Those born Reddish Black approximate in their behaviour closely to a mean represented by the expression (RB→Purple stages→ Int. or Bright Red), and the Intermediate Red to (IR→redder purple stages→Red). What is of main interest is that all the individuals are behaving in very much the same sort of way. This homogeneity is most refreshing.

There are eleven families in the F_6, of which nine are still going and so far have given up to four broods. It is seen that the young at birth are either all Red, or all Intermediate Red ; or a mixture of the two. It is hoped that the homogeneous nature of some of these families is a sign that pure lines are in the process of emerging.

The F_2, F_3, and F_4 families were reared under the incubator conditions, and F_5 and F_6 in the cool. It is impossible to say what difference this has made. At any rate the contrast between the F_3 and F_4 cannot be put down to this account.

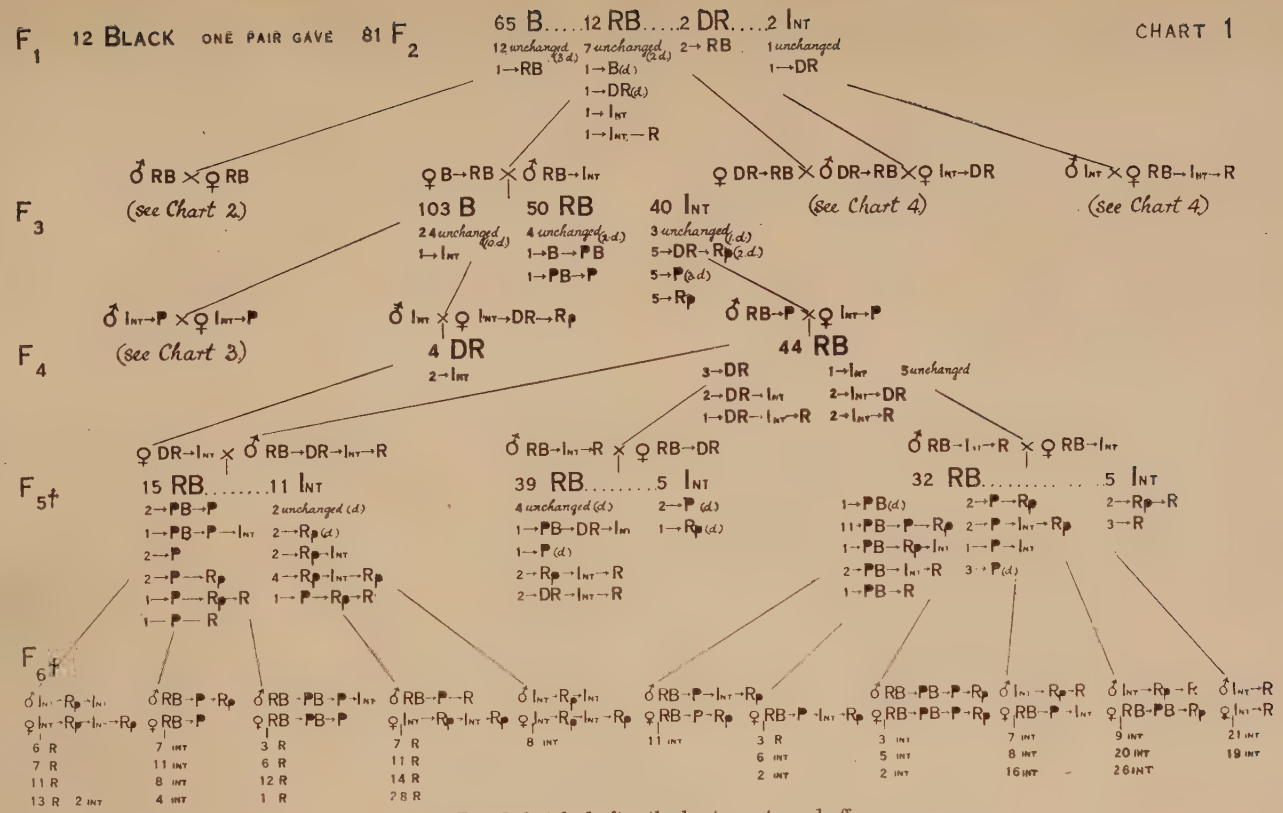
This chart therefore shows (1) the appearance of Purples in the F_3, and how purple parents may give offspring that show no signs of purple ; (2) how more or less homogeneous families are gradually making their appearance in the stock.

Chart 2.

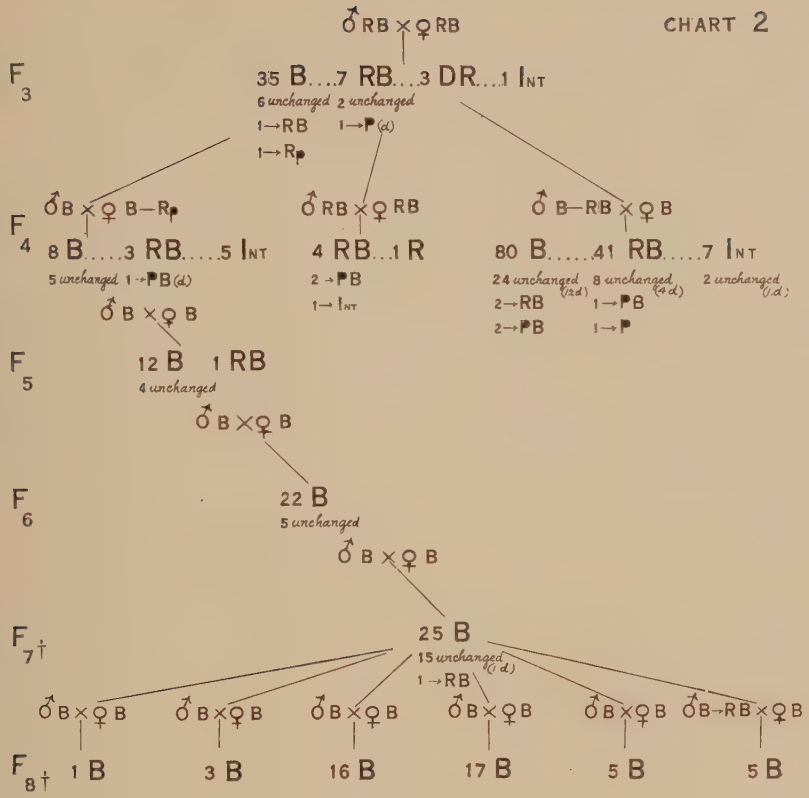
This shows the stage so far reached in an attempt to establish a pure Black stock from an original pair of F_2 Reddish Blacks. Such other matings as gave any result are also included.

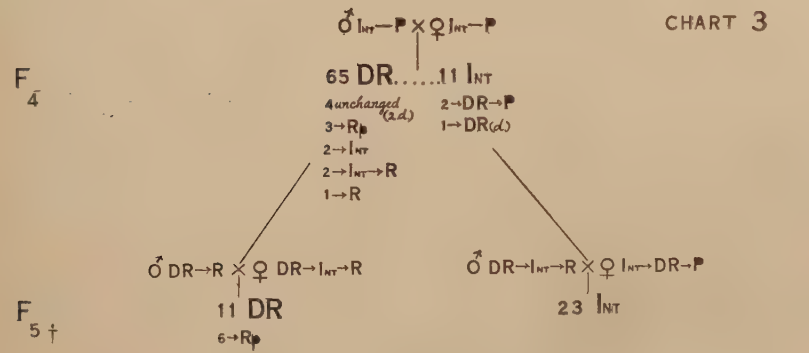
Chart 3.

An early attempt at establishing a Red strain is shown here. It gradually died out. Attention is called to the appearance of Blacks in one of the F_5 families. However, the two survivors did not remain Black ; one changed right over to bright Red, the other to Purplish Black.



† Broods hatched after the heat was turned off.





† Broods hatched after the heat was turned off

CHART 4

† Brood hatched after the heat was turned off.

TABLE II

Summary of Matings in Mutant Stock V

Each rectangle contains the family (or families) resulting from one particular type of mating.
The rectangles are arranged along co-ordinates, each of which represents a type of parent.
The characters of the parents are placed on the left-hand side, the male in all cases given first.
The composition of the family comprises the remainder of the data.
In the case of more than one family from similar type of parents the characters of the latter are not repeated.
The colour given for the offspring is that at birth. Subsequent changes are listed beneath.

The following abbreviations are used :—

B = Black	PB = Purplish Black
RB = Reddish Black	P = Purple
DR = Dark Red	RP = Reddish Purple
IR = Intermediate Red	un. = unchanged
R = Red	(d) = died before maturity

Colour changes are indicated by arrows.

* indicates family still immature at end of May 1930 and in most cases there are more broods to follow.
† indicates hatched and reared at Laboratory Temperature. Others at Incubator Temperature.
Chart complete to end of May 1930.

Chart 4.

Here a small branch derived from the F_3 family set out in Chart 1 is shown. It starts from a mating between two IR→Purple specimens of the F_2 which might reasonably be expected to have been genetically alike. Yet the family produced by them is quite mixed some born DR, some IR ; some turn purple, some redden. The F_5 shows one of the very frequent cases in which purple colour appeared in the progeny of non-purple parents. The other F_5 family is still going and in it so far only Intermediate Reds have appeared.

Table II.

All the matings up to June 1st, 1930, are contained in this table, including several not figured in the previous charts, and many derived from other matings within the F_2. It is set out in table form so as to bring together matings of a similar kind.

It will be noticed that on the whole the mean of the redness of the progeny corresponds to or varies with the mean of the redness of the parents. Thus if the matings of Blacks with various kinds of Red eyes are considered, it is seen that the proportion of Reds in the offspring on the whole increases with the redness of the Red parent. But this does not apply rigidly and scarcely holds at all as far as matings between Reds are concerned.

Again, on the whole, Black is dominant to all the forms of Red and therefore matings between Reds give only Reds. But there are a number of exceptions. For instance, Reddish Blacks not uncommonly give Black among their offspring (e.g. matings between two RB unchanged ; two RB→P's ; RB unchanged×IR→P). Other cases are : a mating between two IR→RB's which gave one Black to 13 Reddish Blacks, and a mating between an IR→RP and DR→RP, to which allusion has been made in Chart 3.

If Black were a simple dominant to all forms of Red, then there would be even chances that a mating between a Black and a Red should produce all Blacks or a mixture of Blacks and Reds. Actually only one out of 19 of such matings has given all Blacks. Even making allowance for a preponderance of heterozygotes, owing to special attention to the redder forms, a higher proportion than 1 out of 19 would be expected.

Value of Data for Genetical Interpretation.

Attention has been called to the small numbers in the earlier broods. Only a few families are large enough to give adequate indications of ratios even of eye characters at birth. (The small number in the broods apparently does not always mean that numbers of eggs die off. It has

been observed that many of the females giving small broods have small ovaries. There is probably not much risk of ratios being upset by a differential death-rate among the eggs.)

But more than this, since the changes undergone after birth are clearly important, it is not sufficient to know the eye-colour of the broods at birth alone. It is only from the survivors that adequate information is to be obtained. The proportion of survivors in the stock has been small, working out at 27%.* That no more than one specimen in three or four survives to maturity is a distinct handicap when so many phenotypes may occur in one family. Recently, however, the proportion has shown a marked increase.

Then there is the question, to which reference has already been made, as to how far the change of temperature has affected the phenotypes.

Finally, attention is called to a rather disturbing feature all too common in this stock, namely, disparity between the composition of and proportions within different broods of the same family. Here is a case in point :

Successive broods from an F_3 DR × B→RB mating had the following composition :

1. 15 IR (11→RB)
2. 15 RB (2→DR, 1 unchanged, 1→IR) ; 2 IR (1→PB).
3. 9 B
4. 12 RB. 10 IR
5. 7 B (2→IR) ; 16 RB (3→IR) ; 4 IR (2 unchanged).

Normally in stocks of Gammarus various phenotypes in a family are fairly evenly distributed among the broods. In this stock there is therefore clearly some irregularity which requires explanation. This at least arouses suspicion of the ratios given by families of only one or two broods.

So far, in spite of the number of families reared, the facts are still inadequate for genetical purposes. No pure lines of any sort of Red eye have yet been produced, and until this is possible there is of course little chance of testing to what extent external factors (in a wide sense) may affect a constitutional type of eye-coloration. For example, throughout the stock there is much variation in the rate of growth, and this variation may well affect the balance between the rate of growth of the ommatidia and the rate of deposition of eye-pigment. Any alteration in this balance means a change in eye-coloration.

Further discussion on the behaviour of this stock and the possibilities

* It is incidentally of interest to note that there has been no marked variation from the 27 per cent survival when the different eye-colours (at birth) are taken separately. Black gives 25%, Reddish Black 27%, Dark Red 32%, and Intermediate Red 29½%. The lower figure for Black, whether it actually means anything or not, might be expected since Blacks occur in larger numbers and hence are kept in more crowded conditions and more at the mercy of cannibals. At any rate, the point is that there is no evidence that the bright Red individuals are less viable than the Blacks.

of genetical interpretation of the results is deferred until additional facts are available. It has been the aim of this paper to give an indication of the problems which have arisen and on which investigation is proceeding.

6. SUMMARY.

For the present, attention is called to the following points:

1. In a large number of experiments with *Gammarus chevreuxi* when the animals were kept under similar conditions at temperatures ranging from 20° to 28° C., twenty instances occurred in which red-eyed mutations appeared independently and simultaneously. The most important of these (Mutant Stock V) is described in detail in this paper.

2. The Red eyes which have arisen in this Stock V and in the four previous Stocks, I to IV, have proved to be all genetically distinct from one another.

3. There are at least two different classes of "red-eye," the one simple, the other with intermediate stages. Mutant Stock V, here described, represents the second type. Differences in the method of origin of the two classes have been pointed out.

4. The inheritance of the red-eyedness of the second class—namely, that in which intermediate stages and various colour changes occur—is of a complicated kind. For an interpretation in terms of Mendelian genes, even if allowance is made for an extent of variation in members of one particular genotype, clearly several of them are involved. Further, there must be considerable interplay of one kind or another among the different genes.

5. Although so many different colour shades have appeared in Mutant Stock V, all can be interpreted in terms of varying concentrations of two pigments—the black and the red. Dilute black pigment with little or no red gives a purple eye.

We are greatly indebted to Dr. E. J. Allen for his constant assistance and advice throughout the whole course of the work; and our best thanks are due to Mr. E. B. Ford of Oxford for the help he has been so ready to give us.

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EXPLANATION OF PLATE VIII.

All the eyes have been figured the same size, so that the colours could be more easily compared.

The colour of each was taken from a living specimen, all the tints in the original water-colour drawings being obtained by using two shades of red with black.

Normal Red from a female of Stock I, sent by Mr. E. B. Ford. This is a pure Red, leaving no deposit of melanin when treated with alcohol, or any other preservative.

New Red from Stock V. A male, H. 814 (2), which was hatched Reddish Black and lightened through Reddish Purple to Red. All these Reds, whether born Red or lightened to Red, contain a slight deposit of melanin, as can be seen on treating them with alcohol. The red pigment, which in life obscures the melanin, dissolves out and leaves a layer of the insoluble dark.

Intermediate Red from a male, H. 813, Pair 1, hatched Reddish Black, and lightened through Reddish Purple to Intermediate Red, and afterwards to Reddish Purple again. This is an example of the fluctuation of colour shown by some specimens.

Dark Red from a young specimen, H. 859b, hatched Dark Red.

Reddish Black from a male, H. 811, hatched Black and lightened to Reddish Black.

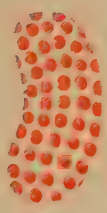
Reddish Purple from a female, H. 844, hatched Intermediate Red, changed to Reddish Purple, then back to Int. Red, and later to Reddish Purple again, another example of the fluctuation of colour through life.

Dark Purple from an immature animal, H. 813, hatched Reddish Black.

Purplish Black, H. 835 a.a., hatched Black, lightened to Purplish Black.

Black from a female, H. 811, unchanged through life.

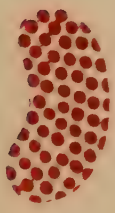
Journ. Mar. Biol. Assoc. XVII.—1.



Normal Red

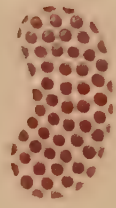
New Red

Inter. Red



Dark Red

Reddish Purple



Dark Purple

Reddish Black

Black

Purplish Black

E. W. Sexton del.

The Cœlenterate Plankton of the Northumbrian Coast during the Year 1924.

By

Ann R. Sanderson, B.Sc.,

University College, Dundee.

With 1 Figure in the Text.

The following is a report on the Cœlenterate plankton taken off the Northumbrian Coast in 1924 during cruises of the *Evadne* of the Dove Marine Laboratory, Cullercoats, Northumberland. The work has been carried out under the supervision of Professor A. D. Peacock to whom I am indebted for much helpful advice. I must thank Professor A. Meek of Armstrong College, Newcastle-upon-Tyne, for so kindly providing the material, and Miss M. Yeats for the use of her notes on the 1924 surface medusæ. Mr. P. L. Kramp of Copenhagen was so good as to check my identifications of certain specimens hitherto unnoted from Northumberland, and for this service I express my obligation. Dr. Allen, Director of the Plymouth Marine Biological Laboratory, and Capt. Totton of the British Museum, have also been kind enough to assist in questions of nomenclature. My own work consisted of the investigation of middle and bottom water samples and Miss Yeats' results on surface samples have also been incorporated here to make the yearly report complete.

Technique. This was the same as that employed by Peacock (see *Dove Marine Laboratory Report*, 1923) and need not be enlarged on here, but a short note on the method of staining may be added. A most useful stain for the medusæ as preserved in the collection* is Picro-Nigrosine, 1 part saturated alcohol solution (70%) stain to 3 parts 70% alcohol. The medusæ are immersed for a short time until they assume a pale green colour. They are then quickly transferred to a differentiating solution of 1 part formic acid and 4 parts 70% alcohol, and left for half an hour. The gelatinous umbrella takes on a bright blue colour and the gonads and manubrium a bright yellow, which colours are intensified on dehydration. This method is particularly useful in examining the oral tentacles of such small specimens as *Lizzia blondina* Forbes. Results were better when a strong solution of Picro-Nigrosine (as above) was used for a short time than when a weaker stain was used for a longer period.

* Fixed in 5% formalin and preserved in 70% alcohol.

The count in each case was absolute for the sample received, for it was found by experiments on estimates made from only a portion of a sample that the enumeration errors were sometimes very large.

The nomenclature employed is that recommended by Mr. E. T. Browne for adoption in the proposed revised list of the Plymouth Fauna.

Notes on the Catches. The most striking feature of the catches is the very great variety of medusæ taken, 22 species in all, of which 17 are confined to middle and bottom waters. In addition to those previously listed for Northumbrian waters there are recorded 9 other species, viz. *Purena* (*Sarsia*) *gemmifera* (Forbes), *Sarsia prolifera* Forbes, *Hybocodon prolifer* L. Agassiz, *Lizzia blondina* Forbes, *Rathkea octopunctata* (M. Sars), *Leuckartiara octona* (Fleming), *Mitrocoma* (*Mitrocomella*) *fulva* (Browne), *Eutonina indicans* (Romanes), and *Æquorea forskalea* Peron et Lesueur.

The numbers of *Obelia* and *Aglantha* are rather small compared with those of former years, but on the other hand this want is balanced by the sudden invasion of the plankton by *Lizzia blondina*, which is found in large numbers at all depths during August and September. While *Hybocodon* is confined to the April catches, *Euphysa*, *Bougainvillia* and *Phialidium* are well represented throughout the whole range.

All the hauls were made in daylight.

Notes on the Species taken.

(For details regarding station, depth and numbers, reference should be made to Table I and Fig. 1. The figures in Table 1 have been arrived at by calculation and express the numbers for the whole catch.)

ANTHOMEDUSÆ.

SARSIIDÆ.

This family, contrary to previous years' experience with similar collections, is well represented. There are at least three different species and, moreover, they illustrate succession in seasonal occurrence. *S. tubulosa* has been found from April to the end of July, then its place is taken by *Purena gemmifera* and *S. prolifera* which continue to be present until the end of October when the catches ceased. The two last-named are listed in this series of Cœlenterate plankton for the first time.

Sarsia tubulosa (M. Sars).

The largest specimen measures about 10 mm. high in the preserved state. The basal bulbs are large and each has a prominent light brown ocellus such as is figured by Kramp (1926, Pt. 10, Pl. I, Fig. 5). The specimens from Stations 2M, 6B and 10M measure 3, 8, and 9 mm. respectively, from which we may conclude, in agreement with Kramp, that

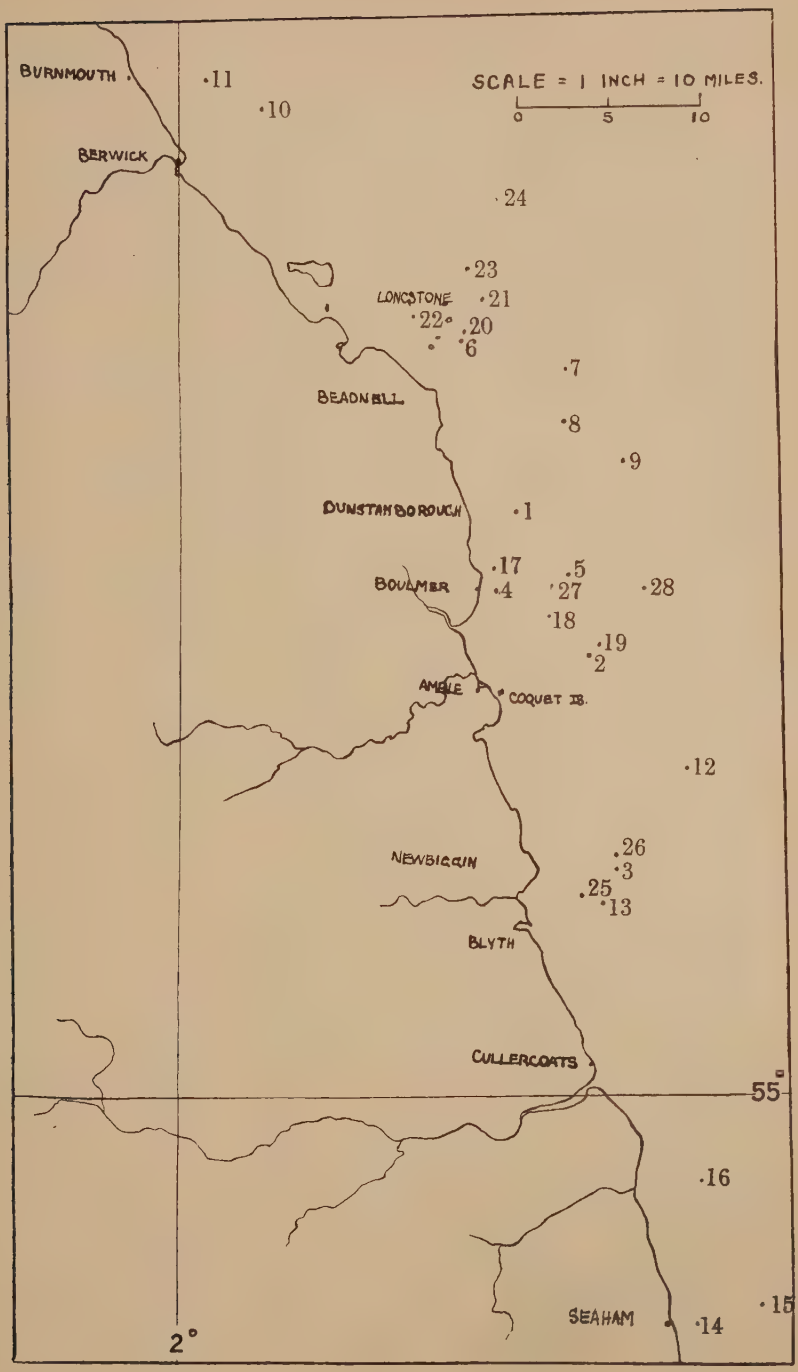

BURNMOUTH
BERWICK
LONGSTONE
BEADNELL
DUNSTANBOROUGH
BOULMER
AMBLE
COQUET IS.
NEWBIGGIN
BLYTH
CULLERCOATS
SEAHAM
SCALE = 1 INCH = 10 MILES.
0
5
10
55°
2°
·1
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Fig. 1.

specimens liberated in April do not reach their maximum size until the end of July. Both *Sarsia tubulosa* (M. Sars) and *S. tubulosa* var. *mirabilis* L. Aggassiz are listed in the 1922 plankton taken in water samples from the Coquet, but Kramp, who has made a very careful examination of the whole *tubulosa*-group (*sensa* Hartlaub), asserts that even in the living specimens he can find no specific differences distinguishing these two. Even if two distinct varieties do exist, I have been unable to determine to which our specimens would belong and have merely designated them *S. tubulosa*.

A few specimens taken are very small, about 1 mm. high, and are sexually immature. The manubrium is without an apical chamber and in some cases is short and slightly swollen above the mouth. In no specimen does the manubrium, even when fully extended, hang below the level of the bell. The tentacles are longer than the bell and are provided with incomplete nematocyst rings. Owing to the state of preservation the ocelli which would furnish a distinguishing character cannot be detected. In general appearance, however, the specimens agree very well with the *Sarsia eximia* figured by Hartlaub in *Nordisches Plankton*. On the other hand they might quite well be very young *S. tubulosa*, for in the latter the manubrium is quite undifferentiated in the young stage. If this is so, then the specimens taken in April would represent the spring brood, from which the large *S. tubulosa* recorded for July have been derived. This possibility is borne out by Kramp's assertion that this species requires $1\frac{1}{2}$–2 months to develop and attain its full size. Moreover, the fact that no large *Sarsia eximia* have been taken seems further to indicate that the specimens in question are young *S. tubulosa*.

Sarsia prolifera Forbes.

In all 71 specimens of this rather rare medusa have been found in the samples examined. All were taken by middle and bottom nets in September and October, with the exception of two which were taken as early as April off Newbiggin. The largest catch was taken on 17th October but all are small, none attaining a height of more than 1·5 mm. Most of the specimens bear buds of various sizes at the bases of the tentacles, and, as those taken in April differ in no way from those taken in September and October, it seems that the species propagates by gemmation during the greater part of the year.

The species, which seems to be confined to the coast of Britain, has been taken by Garstang at Plymouth as early as March and by Mayer at Mousehole as late as November. Those found by the latter author were sexually mature, and, since none of our specimens bears gonads, it seems reasonable to conclude that sexual reproduction is confined to the winter months.

Purena (*Sarsia*) *gemmifera* (Forbes).

This species of Sarsia, which likewise has not hitherto been recorded for these waters, is rather restricted in distribution, appearing only at three stations, one off Cresswell and two in the vicinity of the Boulmer Buoy. The bell varies in height from ·4 to 1·75 mm. and the manubrium when extended may measure as much as 10 mm. The buds in the manubrium do not seem to be developed in any constant order, for in some the oldest primary bud is nearest the proximal end of the manubrium and in others it is second or third in the series. In one specimen the buds in order of size are 2nd, 3rd, 1st, and 4th from the proximal end. Chun, however, has demonstrated his law of succession of buds for this species, and the condition of the above may be explained by the fact that some of these may be secondary or tertiary buds, which first appear on the pedicels of the primary buds and continue to develop after the latter are set free. But, on the other hand, the specimens are so small that I think it unlikely that any primary buds have already been freed. Further, I have not observed any secondary bud developing on the pedicel of a primary one, and therefore I conclude that the law of succession does not hold for this species.

The larger buds also have young buds on the manubrium, but it seems that the primary buds are not set free until they have attained a height of about ·4 mm. This conclusion is based on the fact that none of the medusa buds measures more than ·4 mm. and no free medusæ have been taken in the plankton measuring less than this.

Dipurena (*Slabberia*) *halterata* (Forbes).

Only two specimens were obtained by a bottom net off the Boulmer Buoy in September, one having the manubrium missing. One specimen is about 6 mm. high and has two rings of nematocysts above the terminal bulb of the tentacles, while the other specimen is somewhat higher and the tentacles have three distinct rings of nematocysts above the terminal bulb, the proximal being the smallest and being succeeded by three or four very narrow rings which gradually give place to scattered nematocyst cells.

Euphysa aurata (Forbes).

From the tables it appears as if this medusa has its greatest distribution in bottom waters and gradually shades off in numbers to the surface layers. The largest catches were taken off Burnmouth in July and off Souter in August. The medusæ vary in size up to 2·5 mm. although some are very much shrunken and contracted. There are from 6 to 26 rings of nematocysts in the tentacles, but the majority have about 15. Mayer, in his synopsis of the characters of *Steenstrupia aurata*, states

that there are no definite rings of nematocysts, but Hartlaub, 1907 (p. 83, Fig. 78), gives drawings of tentacles of what he calls *Corymorpha aurata* and *C. nutans*, in which he shows that the former has fewer but broader rings than the latter. (It should be noted that later, p. 87, Fig. 82, Hartlaub uses the name *C. sarsi* for the latter species). Moreover, the specimens, which according to Mayer would be more like *nutans* (Mayer's *Steenstrupia rubra*), from the nature of the tentacle, lack the distinct axial canal which is so characteristic of that species. In some the gelatinous apical projection is fairly well formed, but this character is very variable.

Steenstrupia (*Corymorpha*) *nutans* (M. Sars).

A single specimen, characterised by being larger than the specimens of *Euphysa aurata*, and showing an apical canal and a distinct "shoulder" in the radial canal was classified as *S. nutans*.

Hybocodon prolifer L. Agassiz.

This species has only been recorded from two stations—off Dunstanburgh and the River Coquet. All the specimens which are mentioned show marked asymmetry in the shape of the bell, which varies in height from ·5 to 1·5 mm.

Some have developed two tentacles, but none has three. The large tentacle has about 50 closely-set nematocyst rings, and the lateral tentacle, when present, has about 12. A well-formed lateral bulb may be present on either side of the main bulb, and, as all three bulbs may have secondary buds in some, the cluster is so large as to fill completely the mouth of the bell. The manubrium, which is pear-shaped, can be extended just beyond the edge of the bell, and in many of the specimens it is curved to one side. The mouth is simple and is surrounded by a fairly well-defined rim which according to Kramp bears numerous nematocysts, but these I have been unable to find.

Podocoryne areolata Alder.

This medusa has a rather restricted seasonal distribution, being found only during July and August. The specimens vary in size from ·5–5 mm. and the larger ones have 32 tentacles. Pigment is prominent in the basal bulb and also between the folds of the gonad.

Bougainvillia britannica Forbes.

This species has a regular occurrence throughout the season, but on the whole the numbers taken are fairly small. It is entirely absent from surface layers and the best catch (87) is from a bottom net. The specimens vary in height from 1–8 mm., and, although the evidence is not convincing, yet it is suggestive that the specimens attain their maximum size in July.

Lizzia blondina Forbes.

This is the first record of this species in the Plankton Reports for the North-East Coast, and it is present in such large numbers that it forms the principal part of the medusa plankton catch from August to September. A noteworthy feature is that the medusa has its greatest vertical distribution in surface waters and shows a gradual decrease in numbers towards the deeper water. Further, the hauls taken during its maximum period at stations near the coast, viz. 14, 16, 17 and 22, show a marked decrease in number. It would seem therefore that the medusæ when liberated are carried further out to sea and there find conditions more suitable for a sexual reproduction, by which large swarms can be quickly produced.

The individuals are small, less than 1 mm. in diameter. The bell in the preserved specimens is flattened on top and there are eight tentacle bulbs, the four perradial being in some cases bigger than the interradial and each bearing two tentacles. The manubrium is short and bears several buds on its sides, while the mouth has four simple oral tentacles each ending in a single knob of nematocysts. The distinct oral arms and the grouping of the tentacles show that the medusa belongs to Forbes' genus *Lizzia*. Kramp, following Haeckel, distinguishes three stages in the development of *L. blondina Dysmorphosa minima*, with only eight tentacles, *L. claparedei* with twelve (two on each perradial bulb), and the fully developed *L. blondina* with three tentacles on the perradial and one on each interradial bulb. Our specimens would belong to the two first-named species only, for none has the full number of tentacles and none has gonads. It seems then that the medusa does not become sexually mature till after October, during which month it shows a marked decrease in numbers, possibly owing to the slowing down of the process of gemmation.

Miss Yeats found a small percentage of another medusa, *Willsia stellata* Forbes, but the numbers of these were not determined exactly.

Rathkea octopunctata (M. Sars) (=*Rathkea blumenbachii* Hartlaub).

Only two specimens have been found, one taken by a bottom net and the other by a middle net off Burnmouth in July. They are readily distinguished from *Lizzia blondina* by the light brown pigmentation of the tentacle bulb which carries three tentacles. The manubrium, moreover, has four short stout lips each of which bears two sessile knobs of nematocysts. Just above the base of the lip on either side there is, in addition, another nematocyst cluster. Although the specimens measure only 1 mm. in height, they compare very closely with Hartlaub's drawings in *Nordisches Plankton*.

Leuckartiara octona (Fleming).

This rather striking medusa has a very restricted seasonal occurrence, being found only from August till October, the largest catches being taken by middle and bottom nets. The largest specimen from the Longstone on 11th September measures 10×10 mm. The radial canals are broad and slightly notched and each of the 20 tentacles has an outwardly projecting spur on the adaxial side of the comparatively large basal bulb. The possession of tentacular spurs and a large prominent apical projection immediately points to the specimens belonging to *Leuckartiara octona* (Hartlaub, 1914, p. 285). Kramp has made several observations on number and order of development of tentacles in this species, and on the whole these apply fairly closely to our specimens. The smallest, taken in August off Souter, measures only 1 mm. in height, and has four well-developed tentacles and four interradial rudiments. Judging by its small size we may conclude that it cannot have been carried any great distance, and the hydroid, *Perigonimus repens*, must therefore live somewhere in the neighbourhood.

LEPTOMEDUSÆ.

Mitrocoma (*Mitrocomella*) *fulva* (Browne)
(=*Mitrocoma polydiademata* Romanes.)

This species, listed for this area for the first time, occurs in middle and bottom waters during July. The largest specimen measures 12 mm. in diameter and about 8 mm. in height, and has 42 tentacles with 3 or 4 cirri between adjacent pairs. Lithocysts are present, but I cannot be certain of the number of concretions in each. The manubrium is small and produced at the corners into a slightly recurved lip, a feature which does not seem to have been mentioned elsewhere. In the female the gonad occupies the lower three-quarters of the radial canal and ends within 1 mm. of the edge. A smaller specimen, 7 mm. by 4 mm., has 21 tentacles (5, 5, 5 and 6) and in it the gonad (male) stretches from the circular vessel to the middle of the radial canal. The gonad is relatively larger and more compact than in the female, in which the ova extend in a thin sinuous band on either side of the canal. From an examination of the specimens the males would appear to come to maturity while much smaller than the females.

One specimen, taken off the Coquet (Station 12M.), is peculiar in that two adjacent radial canals, carrying gonads, have swung round and fused about the middle of their course. The common canal formed splits again near the circular canal and gives off a short side branch.

Cosmetira pilosella (Forbes).

This species was taken in very small numbers and was restricted in distribution. The largest specimen, taken off the Longstone in September, measures 13 mm. in diameter, but is sexually spent.

Tiaropsis multicirrata (M. Sars).

In April ten small specimens were taken, the bell in each case measuring about 1·5 mm. in diameter and slightly more in height. There are 32 tentacles with smaller ones developing between them, and eight prominent adradial lithocysts. The mouth is quadrangular with four small lips. No gonads have yet developed.

In July twelve large specimens were taken off Seaham. These vary in diameter from 8–12 mm. and the tentacles vary in number from 176 to 236. According to Bigelow (1913), whose largest specimen measured 22 mm. in diameter, the final number is about 250. In my largest specimens (12 mm.), which are almost sexually mature, the bell margin is closely crowded with tentacles and seems to carry its maximum number, but the actual size of these specimens cannot be determined accurately owing to their preserved state. They differ from the smaller ones in having the bell relatively flatter, the manubrium much more frilled and the gonads well developed and in some cases mature. The collection shows eight females and four males. A prominent feature is the presence of granules of intense black pigment which are scattered along the dorsal wall of the ovary. This pigment is also present at the base of each tentacle, but is only seen clearly in mounted specimens with a fairly high power of the microscope, when the concretions in the marginal vesicles can also be seen to advantage. Owing to preservation these concretions are much broken up and look very like pigment granules. In the male the black pigment is not so conspicuous on the gonad.

The species has been confused with *Eutonina indicans* (Romanes) by Johansen and Levinsen (1903), and indeed the two species are very much alike in the preserved state. As both species were found in the same sample a certain amount of difficulty was experienced at first in distinguishing the two sorts. Mr. Kramp has kindly corroborated these identifications. The peduncle of *Eutonina*, however, although much contracted, is quite distinctive, as are also the black ocelli in the lithocysts of *Tiaropsis*.

Obelia sp.

This medusa occurs in very small numbers compared with those of previous records. Miss Robson has recorded five species of *Obelia* hydroids for the North-East coast (*Dove Marine Report*, 1914), and since there are five medusa-producing hydroids of this genus we should expect to find

five species of medusæ. The work of Browne (1905, p. 70) in rearing different *Obelia* species shows, however, that their medusæ are practically indistinguishable so that, in view of his findings, it does not seem possible to give the specific names of the specimens handled.

Phialidium hemisphæricum (Gronovius).

The numbers of this medusa, present at all stations and at all depths, compare favourably with those of former years. The largest catch was obtained off the Longstone by a bottom net early in September. The specimens range in size up to 12 mm. in diameter and show great variation in form, especially in the number and shape of the tentacle bulbs. Although these differences may be due to the preservative, yet I believe with Kramp that possibly different varieties exist.

One freak specimen has two manubria separated by a distance equal to their own width.

Saphenia gracilis (Forbes and Goodsir).

Although by no means abundant in the collection, this rather rare medusa is present in larger numbers than in previous records. It increases both in number and size from early September to October when the mature specimens are to be found.

Eutonina indicans (Romanes) (=*Eutonina socialis*, Hartlaub).

In all, 33 specimens of this medusa were taken in middle and bottom waters in July and August. They are conspicuous on account of their large size and prominent gelatinous peduncle which bears a much-frilled manubrium. In Hartlaub's figure (1897) this frilled condition is not indicated to the extent shown in my specimens. Kramp has found this species in more northern waters and has made very careful measurements ; with his observations the Northumbrian specimens are in accord.

In the older specimens dark pigment can be detected on the tentacle bulbs, along the dorsal surface of the gonads and along the dorsal surface of the grooves in the floor of the stomach. So far I have been unable to find any reference to this in the literature dealing with this species.

Tima bairdii (Johnston).

Only four specimens were taken in July off the Longstone. All measure more than 20 mm. and one shows traces of a developing gonad.

Æquorea forskalea Péron et Lesueur.

One badly mutilated specimen was taken in July by a bottom net off Seaham. It measures about 12 mm. in diameter and has 16 radial canals. This appears to be the first record of the species for Northumbrian waters.

TRACHYMEDUSÆ.

Aglantha rosea (Forbes).

Compared with the catches of previous years, the numbers recorded for this species are very small. It was entirely absent during July, August and October, and only 25 specimens were obtained during September.

These medusæ do not belong to Kramp's larger form *A. digitale* forma *typica*, but rather to the variety *rosea* for reasons given in a previous Report (Peacock, 1923).

SCYPHOMEDUSÆ.

Aurelia sp.

A single ephyra was captured by a bottom net in April.

CTENOPHORA.

Beroe and Pleurobrachia are well represented from July to October. Beroe is the more numerous for the early part of July, then as it decreases in numbers Pleurobrachia shows a gradual increase. Both are found at all depths.

ACTINOZOA.

Fam. Cereanthidea.

Arachnactis bournei Fowl. (?)

Large numbers of Arachnactis larvæ were taken in April by middle and bottom nets off the Coquet and Newbiggin. They are comparable with the figures of the species given by Carlgren (*Nordisches Plankton*, XI).

Arachnactis albida Sars.

A single specimen of *A. albida* was taken in July. It differs from the former species in the arrangement and length of the tentacles.

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TABLE I.

Species.	Month.	April.					July.								August.			September.						October.				
	Station.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	Depth.*	13	28½	27	19	31	22	34	45	40	33	33	39	34	12	28	25	18	26	39	22	38½	11	41	35½	24	29	27½	37½
Sarsia eximia	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	2	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	5	–	7	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sarsia tubulosa	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	2	–	–	–	–	–	–	–	2	–	–	–	–	2	2	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	4	–	–	2	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sarsia prolifera	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	7	–	12	52	9	4
	B	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–	–	–	8	43	5	2
Sarsia gemmifera	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	4
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	25	–	–	–	–	–	–	–	–	2	–	–
Dipurena halterata	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–
Euphysa aurata	S	–	–	–	–	–	59	–	–	–	2	–	–	–	–	–	–	–	43	–	2	9	–	2	–	65	25	–	–
	M	5	–	–	20	2	12	66	81	56	66	43	5	–	9	57	57	4	4	–	8	–	–	12	11	4	7	9	14
	B	7	2	–	7	26	40	95	221	122	499	437	55	24	46	167	549	–	10	6	4	–	–	6	35	4	2	10	27
Hybocodon prolifer	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	12	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Podocoryne areolata	S	–	–	–	–	3	11	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	2	4	–	2	7	7	5	10	22	–	–	70	24	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	6	–	11	2	3	5	–	27	36	2	3	8	–	–	–	–	–	–	–	–	–	–	–	–
Bougainvillia britannica	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	5	–	–	–	7	9	14	7	–	7	–	–	9	6	–	–	4	–	–	–	2	4	–	–	–	–
	B	–	–	2	–	3	–	–	9	2	–	–	87	17	–	3	8	–	2	–	–	–	–	4	–	–	2	–	–
Lizzia blondina	S	–	–	–	–	–	–	–	–	–	–	–	8	–	–	528	5	–	23,400	9,572	8	1,092	–	68	5,152	–	–	–	–
	M	–	–	–	–	2	–	–	–	–	–	–	–	–	4	1,431	102	68	968	759	164	13	59	493	3,676	6	16	–	–
	B	–	–	–	–	–	6	–	–	–	–	–	–	–	4	235	40	18	666	1,172	73	2	36	117	376	18	6	–	–
Rathkc octopunctata	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Leuckartiara octona	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	17	–	–	2	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	2	7	–	18	4	2	7	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	12	–	6	2	2	–	–	–

* Depth of sea bottom in fathoms.

TABLE I.—*continued.*

	Species.	Month.	April.				July.								August.				September.						October.			
		Station.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
		Depth.*	31	28½	27	19	31	22	34	45	40	33	33	39	34	12	28	25	18	26	39	22	38½	11	41	35½	24	29	27½	37½
Leptomedusæ	Mitrocoma fulva	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	–	–	–	–	–	–	–	–
		M	–		–	–		–	–	7	11	2	–	7	–	–		–	–	–		–		–	–	–	–	–	–	–
		B	–	–	–	–	6	2	–	2	–	3	–	5	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Cosmetira pilosella	S	–	–	–	–	–	–		–	–		–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	–	–	–	–	–	–	2	–
		B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	2	4	–	–		4	–	–	–	–	–
	Tiaropsis multicirrata	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		M	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		B	–	7	3	–	–	–	–	–	–	–	–	–	–	23	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Obelia sp.	S	–	–	–	–	5	–	–	2	–	17	2	22	18	–	20	–	–	–	11	2	–	–	–	–	–	–	–	–
		M	14	–	16	–	20	–	14	2	2	2	–	–	33	–	–	2	2	–	–	4	–	–	3	2	–	–	–	–
		B	–	5	2	–	6	–	–	2	6	11	–	–	12	–	3	–	–	–	–	–	–	–	4	–	2	2	4	–
	Phialidium hemisphæricum	S	–	–	–	–	5	55	–	–	–	–	2	15	2	28	4	17	–	–	4	18	–	18	–	4	–	–	–	–
		M	–	–	–	–	40	3	23	51	23	59	40	54	13	72	20	89	10	–	2	14	–	5	64	21	32	5	22	58
		B	–	3	2	7	87	4	43	155	15	35	23	33	136	119	18	38	46	16	11	5	2	4	119	469	21	30	7	46
	Saphenia gracilis	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	8	–	2	7	9	10	–	25	12
		B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	4	–	4	9	6	5	13
	Eutonina indicans	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		M	–	–	–	–	–	–	–	7	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		B	–	–	–	3	–	–	–	4	4	–	–	38	–	2	12	–	–	–	–	–	–	–	–	–	–	–	–	–
	Tima bairdii	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		B	–	–	–	–	–	–	–	4†	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trachymedusæ	Aglantha rosea	S		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	2	–	2	–	6	–	–	–	–
		M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	6	–	–	–	–
		B	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

* Depth of sea bottom in fathoms. † Absolute no.

The Cœlenterate Plankton of the Northumbrian Coast during the Year 1925.

By

Henry G. Watson, B.Sc.

With 1 Figure in the Text.

This is a report of the Cœlenterate plankton taken during the cruises of the *Evadne* in 1925, and investigated at the request of Professor A. D. Peacock. I am indebted to Miss Ann R. Sanderson, B.Sc., for considerable help in the identification of specimens.

The material was received from the Dove Marine Laboratory already separated from the whole catch of the plankton. In most cases only a fraction of the catch had been examined, but the numbers in the table express the total for the whole catch. Instead of hauls being taken at three depths as in previous years, hauls were taken at five depths, but the "mid-over" position was discontinued after April. All the hauls were made in daylight.

Reviewing the estimated numbers taken, it appears that on the whole the cœlenterates were not so abundant in 1925 as in previous years, *Hybocodon prolifer* L. Agassiz, and *Aglantha rosea* (Forbes) being the only species whose numbers compare at all favourably with those of former collections. Seventeen species were noted, seven occurring in middle and bottom waters only. No new species was found.

Cœlenterates were absent from several hauls taken in April, namely, off Beadnell (Sta. 1 surface, Sta. 2 surface) and off Coquet (Sta. 9 surface and mid-over).

For details regarding station, depth and numbers reference should be made to Table I and Fig. 1. The figures in Table 1 have been expressed proportionately in terms of the whole catch.

ANTHOMEDUSÆ.

Sarsia eximia Allman, and *S. tubulosa* (M. Sars).

In all only five specimens of these were found and each number in the table represents only one specimen. In the case of each *S. eximia* specimen, it was so badly mutilated as to be identifiable with difficulty.

Euphysa aurata (Forbes.)

This was common in all localities in June and July, with a tendency to be found in the lower hauls. It was notably absent in September, a similar observation being made in the Reports of 1922 and 1923. A catch far greater than any other was taken off Newbiggin (Sta. 18 Bottom) on July 11th. *Steenstrupia nutans* (M. Sars), if present, was not identified.

Hybocodon prolifer L. Agassiz.

This was far more numerous at the two stations established on June 1st, one off Newbiggin, the other off Coquet, than at any other time and in each case in the middle hauls. It was rare in April and almost absent in the catches later in the year.

Podocoryne areolata Alder.

Only isolated specimens of this medusa occur.

Bougainvillia britannica Forbes.

This was found at all depths in June and July, but was entirely absent in April and September. The best catch was taken of Dunstanborough (Sta. 16), the middle haul being the largest. It may be noted that it was absent off Boulmer (Sta. 15) on the same day. The total of all specimens of medusæ from Station 15 is remarkably small.

Lizzia blondina Forbes.

The occurrence of this species was erratic, being found only at a few stations in small numbers at all depths. Its occurrence this year bears no resemblance to that of 1924.

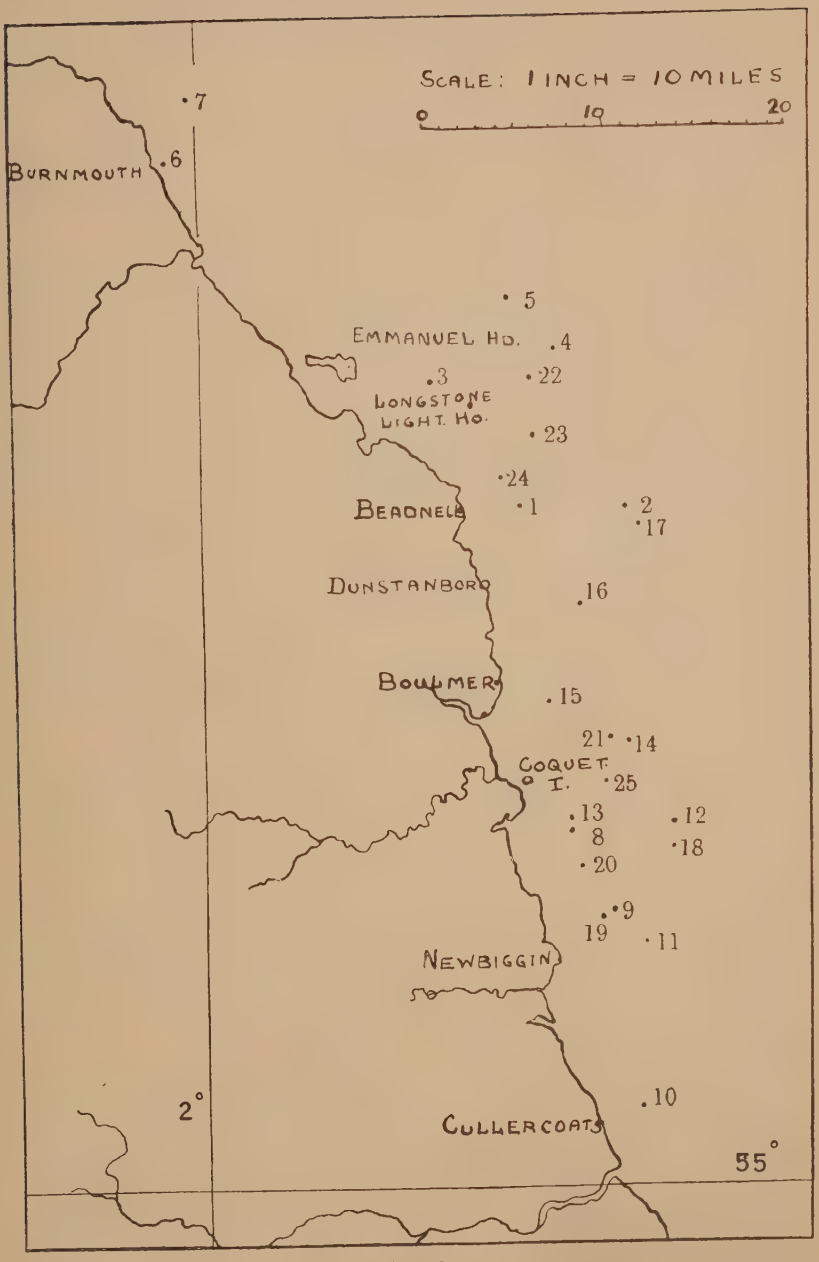
Rathkea octopunctata (M. Sars).

This uncommon species was a little more frequent in its appearance than in the previous year. The identification of each specimen was confirmed by the character of the oral tentacles.

LEPTOMEDUSÆ.

Mitrocoma fulva (Browne).

This occurs in middle and bottom waters in April, June and July. A single specimen was found in the fraction from a surface haul taken off Alnmouth in September (Sta. 21). Its occurrence appears unusual.


SCALE: 1 INCH = 10 MILES
0
10
20
BURNMOUTH
EMMANUEL HD.
LONGSTONE LIGHT. HO.
BEADNELL
DUNSTANBORO
BOULMER
COQUET I.
NEWBIGGIN
CULLERCOATS
2°
55°
.7
.6
.5
.4
.3
.22
.23
.24
.1
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.16
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.19
.11
.10

FIG. 1.

Cosmetira pilosella (Forbes).

The single specimen obtained in April is doubtfully identified, being shrunken and much stained. The species occurred in small numbers in September in the lower waters only, the specimens being large if not mature.

Tiaropsis multicirrata (M. Sars).

This species occurred again this year, but in small numbers. It appeared as last year, in April and not again until the latter part of July. The specimens obtained in April were small, about 1 mm. across, while those in July were almost, if not, mature.

Obelia sp.

The difficulty of distinguishing the medusæ of the various species has caused them to be numbered together. The numbers are a slight increase on those of the previous year, except in April, and they compare favourably with those of 1923.

Phialidium hemisphæricum (Gronovius).

The numbers of this medusa are less than in any of the two previous years. It was present at all depths with a tendency to be found in the lower hauls, a similar observation being made in previous years. Specimens of all sizes occurred throughout the whole collection.

Eutonina indicans (Romanes).

A total of 8 medium-sized specimens was obtained from three stations, namely, St. Abb's Head (Sta. 7), Newbiggin (Sta. 11) and Coquet (Sta. 14).

Tima bairdii (Johnston).

One specimen only was obtained, in the middle haul off Boulmer (Sta. 15).

TRACHYMEDUSÆ.

Aglantha rosea (Forbes).

This was by far the most abundant species. Though notably absent entirely from two stations (Burnmouth, Sta. 6, and Coquet, Sta. 12) it occurred in almost every one of the other hauls at all the other stations. It was equally common at all depths. The numbers seem to reach a peak at the beginning of September, though the largest catch by far was taken at the last station. The numbers compare favourably with those of other years.

TABLE I.

Species.	Month.	April.						June.					July.						September.						
	Date.	8	8	10	10	10	11	11	14	14	14	1	1	10	10	11	11	11	11	7	7	7	13	13	13	17
	Station.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Depth.	30	38	10	47	39½	22	37	24	32	20	27	30	25	30	21½	26	41½	25	22	30	30	32	29	24½	29
Sarsia eximia	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sarsia tubulosa	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	1·5	19	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–
Euphysa aurata	S	–	–	–	–	–	–	–	–	–	–	–	3·5	8·75	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	1·8	–	–	–	23	–	–	–	–	–	57	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	2	–	–	2	–	4	2	4	–	–	16	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	1·7	–	2·2	1·5	–	9·3	8·8	–	8·8	–	120	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hybocodon prolifer	S	–	–	–	–	–	–	–	–	–	–	3·5	10·3	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	1·8	29·9	4·6	–	–	–	–	6	–	–	–	–	–	–	–	–
	M under	1	–	–	–	–	3	–	–	–	–	13	–	2	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	2	–	2	5	–	–	2·2	10·8	–	–	5·5	–	–	–	–	–	–	–	–	–	–	–
Leuckartiara octona	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7·5	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	34	11·3	–	–	–	2·1	9
	M	–	–	–	–	–	–	–	–	–	–	2	–	–	4	–	–	–	–	135	414	46·7	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13·7	16	–	–	–	–	–
	B	–	–	–		–			–	–	–	–	–	–	–	–	–	–	–							

TABLE I—*continued.*

	Month.	April.						June.				July.						September.						
	Date.	8	8	10	10	10	11	11	14	14	14	1	1	10	10	11	11	11	7	7	7	13	13	17
	Station.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Species.	Depth.	30	38	10	47	39½	22	37	24	32	20	27	30	25	30	21½	26	41½	25	22	30	30	32	29	24½	29
Podocoryne areolata	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2·75	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	1·7	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	38	–	–	–	–	–	–	–	–	–	–	–	–	–
Bougainvillia britannica	S	–	–	–	–	–	–	–	–	–	–	–	–	–	4·8	–	16	–	8	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	6·4	–	6·2	16	–	41·3	18	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	8	–	2	4	–	20	5·1	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	7·7	76	–	–	–	13·2	12·7	–	–	–	–	–	–	–	–
Lizzia blondina	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26·3	24	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	16	–	–	–	9·5	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	16	20·6	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	19	–	–	–	–	–	–	–	–
Rathkea octopunctata	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	3	–	–	–	?	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	6·2	–	9·3	–	–	–	–	–	–	–	–	–	–	–	–
Mitrocoma fulva	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–
	M over	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	8	4·3	–	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M under	–	–	–	5	–	–	15·8	1	–	–	2	–	–	2	–	2	–	–	–	–	–	–	–	–	–
	B	–	–	–	2	–	–	5	1·7	1·9	–	6·2	38	–	–	–	–	–	–	–	–	–	–	–	–	–
Cosmetira pilosella	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7·8	–	19·1	–
	M under	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	21	8·3	6	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20·5	–	–	26	–	10	–

LEPTOMEDUSÆ.

Tiaropsis multicirrata	S	–	–	–	–	–	1·8	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–
	M over	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9·5	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
	B	–	1·5	–	–	–	–	–	–	–	–	–	–	–	–	–	4·4	–	–	–	–	–	–	–	–	–
Obelia sp.	S	–	–	–	–	–	–	–	–	–	–	56	75·3	140	33·6	–	–	64	52	5	–	10	15	3·1	3·9	114
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	8·5	218·5	–	–	–	–	6	38	5·7	–	8·3	–	–	4·2	–
	M under	–	–	–	–	3	–	–	–	1	–	11	66	6	18	–	4	3·4	4	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	10·8	–	–	23·3	–	–	19	–	–	–	–	19·5	–	–	–
Phialidium hemisphæricum	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6·8	–	–	16	5	–	–	–	3·1	–	12
	M over	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	1·8	–	–	–	2·1	–	–	–	–	–	–	19	–	–	16·7	–	–	4·2	–
	M under	–	–	–	–	–	3	4·5	1	1	–	–	–	4	20	4	2	–	4	4·5	4·6	–	–	–	–	24
	B	–	–	–	–	–	–	11·8	–	–	–	1·5	19	9·3	5·8	–	–	31·7	40	–	10·7	21·4	39	–	2	21
Eutonina indicans	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	4·5	–	–	–	3	–	–	4	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	5·8	–	–	–	–	–	–	–	–	–	–	–
Tima bairdii	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M over	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5·6	–	–	–	–	–	–	–	–	–	–
	M under	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aglantha rosea	S	–	–	–	1·7	–	–	2	–	–	–	–	–	–	33·6	–	10·5	4	48	125	144	10	382·5	40·4	11·7	192
	M over	–	9	–	12	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	M	3·5	11	2·5	28	2	–	1·8	10·8	7·2	9	10·7	–	105·8	32	5·6	2·8	12	114	317·3	236·3	108·7	317·8	91·7	14·8	792
	M under	1	1	–	5	–	–	–	4	–	1	6	–	58	12	4	2	3·4	66	209·3	377·2	33·3	364	no catch	–	416
	B	–	4·5	–	–	–	–	1·7	–	3·8	2·2	6·2	–	28	17·5	–	–	6·3	200	416·8	346·7	92·9	318·5	60·7	10	294

TRACHYMEDUSÆ.

Further Investigations upon the Water Movements in the English Channel.

Drift-Bottle Experiments in the Summers of 1927, 1928 and 1929, with Critical Notes on Drift-Bottle Experiments in General.

By

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With 4 Charts and 2 Figures in the Text.

As long ago as 1897 the Marine Biological Association carried out experiments with surface-floating drift bottles in the English Channel. In the course of those experiments which were described by Garstang in this Journal (**1**), 430 bottles were sent adrift in 53 batches during the year 1897. The bottles were put out chiefly in the vicinity of the Eddystone, and when Garstang reported on the results of the experiments, rather less than one-third of the bottles had been recovered. Garstang worked out a relationship between the movements of his bottles and the strength of the winds, which convinced him that "the relation between wind action and surface currents is capable of quantitative study," and declared himself encouraged to proceed further with the idea of testing the reliability of his empirical relationship between bottle travel in miles and wind *pressure*. The study of wind influence by paying attention to *pressure* constituted a step of interest, and one would like to be able to turn up the continuation of Garstang's work promised at the conclusion of his paper already quoted. We here pay more than passing attention to the attempt made by Garstang to establish a quantitative relationship which should enable one to infer surface current speed from wind, because it is our intention to examine this matter further at a later stage in this paper. For the present, one may remark that any investigator who may seek to connect wind and surface drift by means of a quantitative relationship wherein the wind factor employed is wind velocity squared, is, to all intents and purposes, expressing wind influence in terms of pressure like Garstang.* We shall refer to this worker's results again later.

In 1925 a very brief paper on the movements of surface-drift bottles put out from the Sandettie Lightship during a whole year (1920–21) was

* See page 40 of *The Observer's Handbook*—M.O. 191.

published in this Journal by the present writer (**2**). This small paper was written to throw what light could at the time be thrown upon the question of water interchange between English Channel and North Sea. Actually at the present time this is a subject upon which we have ample information from sources other than drift-bottle experiments.

In 1927 a further paper dealing with drift-bottle experiments in the English Channel appeared in this Journal (**3**), and to certain results therein set down some later reference will be made.

In view of the fact that these three papers have been published in this Journal, it is convenient that the present paper should find a place here.

It is our intention to present below the results obtained from the setting adrift of numbers of bottles (not all of the same kind) from the International Station E2, situated at 49° 27′ North -4° 42′ West. The bottles were put out from the Ministry's research vessel *George Bligh* by the writer's colleague Mr. C. F. Hickling, in early August 1927, in August 1928, and in July 1929.

The experiments of July 1924 had involved the liberation of 500 bottles—of which half were common surface-floating bottles and half bottom-trailing bottles of Bidder type ; in that case liberations of 50 of each kind were made at International Station E2, at International Station E3 (48° 34′ North—5° 13′ West), and along three chosen short stretches on the steamer route between Southampton and Saint Malo. From these earlier experiments (1924) 52 per cent of the surface bottles were recovered, and much information of interest resulted.

In the case of the experiments with which we are to be concerned below, no bottom-trailing bottles call for our attention ; the liberations were made only at E2, and that on one occasion only in each of the three years in question. However, the experiments were made for very definite reasons which will become apparent later. There was, of course, the obvious general wish to find out more about the surface-water movements of the English Channel in order to amplify what had already been discovered, but, since we had in 1924 been particularly fortunate in choosing what proved to be an unusually interesting time for our experiments, we now wished to repeat these to see what journeys might *again* be accomplished by ordinary surface bottles. Subsequent to the 1924 liberations, there had been an onset of S.W. wind of almost uninterrupted predominance for some 5½ months ; some of the common surface bottles had travelled about 500 miles at an overall speed approaching 8 miles a day, a speed which we naturally surmised might be unusually great. This, amongst other reasons, led us to repeat the experiment. Another reason was the wish to see whether we could not obtain useful and representative data to enable us to work out on better foundations than had yet been possible, a reliable quantitative relationship between surface

water movement and wind strength. On this point we have elsewhere indicated what we hold to be representative data (**3**, p. 717). The third, and chief reason, arose from our desire to try out other types of bottle, and to stage a race as it were between these latter and ordinary surface-floating bottles. Added to the foregoing reasons was the very natural wish to observe at what speed bottles travel up Channel, at a time during the whole of which we have records of the speed of water flow through Dover Straits. When the earlier drift-bottle experiments were made in the Channel we had no continuous current observations in train from the Varne Light vessel, such as are now available.

The English Channel is, of course, an ideal place for carrying out experiments with different types of floating drift bottles. Bottles may travel 1000 miles and more with very good chances of recovery when they strand ; no matter where they may go ashore short of this distance, the prospects of receiving the postcards from them are quite good.

General Remarks on Drift Bottles and their Use.

By our expression " other types of bottle " we mean various kinds of floating-bottle systems provided with sub-surface drags. The shortcomings of simple surface-floating bottles have been apparent to earlier workers, of course, and bottles fitted with suspended objects of various kinds have been used.* The writer has devised various types of fittings to enable drag bottles to be made up satisfactorily and quickly ; one of these has been described and figured already (**4**), whilst others are pictured in this present paper. Now one point concerning the employment of drag-fitted bottles is of supreme importance, but strangely enough there seems to be little indication that all earlier workers have paid proper attention to it. It is all very well to put out drag-fitted bottles, but unless one can have very definite information that the postcard bottle had actually stranded *complete with fittings exactly as when set adrift* at the place wherefrom the postcard was sent back, one surely has no option but to discard the postcard as almost worthless. This is certainly the considered opinion of the writer who has carried out various experiments to evolve a really successful drag-fitted bottle. In the course of drift-bottle experiments in the Adriatic, Italian workers did take steps to learn whether the entire floating system stranded at the place from which the postcard was returned.

A few general considerations will repay attention at this point. If ordinary surface-drift bottles move (as they have been known to do) up the English Channel into the North Sea at an average speed exceeding 6 miles a day (**3**), we may not assume that there has been any commensurate translocation of water unless we have special evidence of this.

* References are given later.

Such evidence might be available from the study of changing salinity distribution. As Harvey (**5**, p. 103) so rightly remarks, "it appears probable that the wind-blown surface particles, after travelling a short distance, sink, to be replaced by particles from below, and in this way translocation of water masses with particular characteristics, containing characteristic organisms, does not take place at anything approaching the speed of a floating object."

It is surely high time that the implications of these considerations should be fully weighed. All the passively conveyed organisms whose movements in the sea interest us fishery researchers, are so nearly of the same specific gravity as the water itself, that we cannot refuse to entertain the possibility that they may undergo passive vertical movements in the upper water layers of equal magnitude with their passive horizontal movements. An object like a simple floating bottle (even when perfectly properly ballasted to float correctly) escapes the vertical movements referred to, and will move along at a rate approaching the onward movement of individual water particles as impelled by the wind during their brief sojourn at the surface. Now without going too fully into the matter, it is at any rate clear that a system comprising a buoyant bottle from which a drag is suspended, will move forwards at a rate much more nearly approximating to that of planktonic organisms in the same milieu ; *in cases where the water movements can be regarded as wind-impelled* an important reservation. Just how badly out (if errant at all) are the results obtained with simple surface bottles in an area like the southern North Sea, where wind plays a potent rôle in influencing waters constantly moved to and fro in immense masses by the swing of the tides, the writer is not prepared even to attempt to indicate here. Of course, the remarks just quoted from Harvey demand fuller attention in some cases than in others. Imagine a large still lake having no outlets and no noticeable currents. Let us put out in such a lake a number of simple surface-drift bottles and await the onset of strong persistent winds. We shall evince no surprise if the bottles (though properly adjusted to float only just awash) move away down wind at a speed very much greater than does, say, the centre of mass of a population of plankton which we may earlier have discovered to be distributed throughout the upper layers where we put out the bottles. This is a simple case. In a sea affected by strong tidal streams the question is very much less simple. It may be that on days without wind the sub-surface tidal streams are, at places, faster than are the surface streams. In the case of the English Channel at times of strong southwesterly winds, rapid North Sea-going travel of simple surface bottles may be attributable to the wind hindering the bottles moving back again on the southwest-going streams. Thus all sorts of complications arise ; a certain strength of wind in one direction may be

much more potent than the same strength of wind in the opposite direction in influencing the travel of surface bottles, due to unequal speed of the streams opposed. To what degree the ideas so clearly applicable to waters free of tidal streams apply, is a matter for experimental elucidation.

Granted that it is desirable to employ drag-fitted bottles, how best may we construct such bottles? It is obviously desirable that the drag unit should hang as vertically as possible below the buoyant floating unit. This implies that as much weight as possible should reside in the drag to prevent it from riding up, for, in a sea where the water movements are wind impelled, we desire by the very nature of things that the drag should always hang as deep as possible. This desideratum requires that the upper floating bottle shall be as buoyant as possible so that it shall be able to support a drag of maximum weight. In turn this demands that the suspending line or wire shall be good and strong, and that its attachments to the two constituent units shall be above suspicion. All these features go to make a satisfactory system, but, as an offset, they impose a need for great caution in making safe deductions from the use of floats embodying them. It is clear that such a bottle system as meets all the requirements outlined above might float in good style from E2, say, as far as the Straits of Dover, whereupon it may elect to strand. It will become moored in a fathom of water perhaps, and the constant moving about and battering it may be subjected to there can very easily be expected to cause a breakage adrift as between bottle and drag. What happens now? The buoyant bottle receives a new lease of life as it were, and may easily move offshore again for any one of a variety of reasons. It is now almost completely useless as an indicator of surface-water movements, for it will be blown about by the wind from place to place, being entirely unballasted. It may ultimately strand and be found, say, in Jutland, in which case its travel might be taken without comment as in no way amiss. It might on the other hand strand on the Yorkshire coast, when one would be presented with the choice between inferring an interesting because unusual drift, and the possibility of something being very much amiss. A period of southeasterly winds could (and has been known to) cause the upper unit of a drag-bottle system to travel to the Yorkshire coast after having come from E2 to the North Sea with nothing wrong with the system. All these considerations imply that we must have a good drag-bottle system so designed that we shall know whether *all* was present when the questionnaire bottle stranded. We shall feel inclined to appraise earlier work involving the use of drag-fitted bottles in the light of the foregoing considerations.

Relevant Comments on the Views held by, and the Apparatus employed by Earlier Workers with different kinds of Drift Bottles.

The literature concerning drift-bottle experiments is already very considerable, and it can be no aim of a paper such as this to attempt to give an adequate account of the actual apparatus employed by different workers except in such cases as have a particularly close bearing on matters here dealt with. The first point of interest, of course, is the importance attached by earlier workers to the necessity of using bottles very carefully ballasted to escape windage effect. Let us see what degree of reliance investigators have found it possible to place on the travels of simple surface bottles regarded as true indicators of surface currents. Thereafter will come the wish to learn what views have been held by earlier workers regarding the desirability of employing bottles fitted with sub-surface drags.

It is definitely a fact that there exists a cleavage of opinion. Krümmel (**8**) discusses the earliest-known drift-bottle experiments and weighs the opinions as to their usefulness held by various authorities. He declares his own opinion (**8**, p. 437) thus : " Wir wissen jetzt, dass die Flaschen auch ohne einen besonderen kleinen Ballast von Sand tief genug eintauchen (sie werden meist mit der Zeit immer schwerer durch Bewachsung), um überwiegend dem Strom, nicht einfach dem Winde zu folgen : ganz abgesehen davon, dass in der offenen See die Richtung der herrschenden Luft-und Meeresströmungen nicht eben sehr verschieden zu sein pflegt."

That drift bottles may move against the prevailing wind even when the current is weak and whether they are ballasted or not has been proved by Schott especially (**9**). Ryder (**10**) was content to use ordinary champaign [*sic*] bottles without ballast ; these we are told floated horizontally in the water but with only a very small part of their side above water, " so that they were practically inexposed to the action of the wind." Nielsen (**11**) attached great importance to using bottles carefully adjusted to approach very nearly the specific gravity of the water. Schmidt (**9**) apparently used ordinary champagne bottles, some ballasted to float with only a very little surface exposed, and some unballasted. He intended, however, to ascertain whether the two types performed journeys significantly different, and promised a discussion in the second report on the drift-bottle experiments, but in this report written by Giovanni Platania (**12**) no such discussion appears. Gilson (**13**) in his very fine and detailed report discusses in detail the advantages of using bottles other than simple surface floaters. Incidentally we learn (**14**) that his report only presents a quarter of the material at his disposal for publication. The surface-drift bottles used by the English Ministry

of Fisheries (**7**) were all of a type carefully shingle ballasted to float vertically with the tips of their necks only just awash (**15**). In these experiments the bottles certainly did not experience a direct wind pressure, but their travels were conspicuously in accord with the direction of the prevailing winds, a fact which makes one wish very strongly that contemporaneous liberations of drag-fitted bottles had been possible, for it is fair to suppose that such a large-scale bottle experiment will never be repeated —indeed, two of the lightvessels from which the bottles were then set adrift are no longer in existence.

The foregoing remarks serve to show that various opinions have been held as to the necessity of carefully weighting simple surface bottles, but it is surely a very elementary precaution to take, and the present writer (who gives experimental findings bearing on this matter below) certainly holds the view that it is a necessary step to take, being convinced that the strictures made by Sir John Ross so long ago (see **8**, p. 437), when he spoke of the "bottle fallacy," could be levelled with good reason against results obtained from the use of bottles not weighted to ride with practically all their surface submerged.

There can be little doubt that it is much better to use bottles fitted with suspended sub-surface objects; though they are less easy to make up and use in large numbers, their superior usefulness can hardly be challenged. That many workers have held this view is quite clear, and it certainly represents the majority opinion of most marine researchers with whom the writer has spoken. A well-ballasted simple surface floater may certainly be taken along by the current in spite of the prevalence of a strong opposing wind, but it is a matter of experience that if one chooses an occasion when a pronounced surface stream is opposed by a strong wind to put out bottles of both kinds, one will soon see that the bottles separate considerably. This is a fact not to be neglected, and it can with reason be urged that the travels of a bottle system fitted with a drag of adequate surface hung, say, a fathom deep, are likely to give to the marine fishery researcher indications of superior value to the travels of simple surface-floating bottles.

Admittedly a most obvious advantage of the simple surface floater resides in the fact that with it, we are free from the risk that a large proportion of our returned postcards may be useless as a result of ignorance as to whether the bottles when they stranded were exactly as when set adrift. Drag-fitted bottles have been used by various earlier investigators, though reference can here be made only to such experiments as are of special interest to us. Garstang realised the desirability of experiments to determine the depth of the currents induced by wind action (**1**, p. 225) and envisaged a comparison of results obtained by bottles floating at the surface, and by other objects designed to come under the influence

of lower strata of water. He appears, however, to have reported upon no experiments to the end outlined. Cunningham in a paper rarely quoted (**6**), in the third year of his drift-bottle experiments in the Irish Sea, continued his liberations of simple surface-floating bottles, "but side by side with it other schemes were tried with a view to ascertain the movements of the deep water. 'Vehicles' of various form were designed, bamboo and wood being used in their construction; some were weighted with lead to make them sink, and others left without any ballast to float lightly on the water."* It is not possible to learn all one would like from Cunningham's paper, and he gives no wind data.

References to work with drag-fitted bottles are given by the writer elsewhere (**7**, p. 7). In Gilson's well-known experiments (**13**, p. 7) coupled bottles were used. He deals fully with the reasons for using coupled bottles, describes his apparatus in detail, and gives an account of instances when he has noticed a marked difference in rate of travel in the southern North Sea as between simple surface-floating bottles and his drag-bottle systems, consisting of pairs of bottles linked together by cords 3 metres long. When wind and current are in the same sense the simple floater quickly races the other; where opposed the reverse is the case. Gilson was aware when his postcards came back whether his coupled bottles had been found still fastened together, and realised that the upper buoyant members of his systems, in cases where the suspending cords had broken, were "beaucoup plus aptes à être emportés par le vent." He cites cases where simple bottles had travelled at 13½ miles a day, whereas their fellow coupled bottles had, under the same favourable wind conditions, travelled at only 6 miles a day. Again, he gives instances where simple floating bottles and coupled bottles put out at the same time have performed significantly different journeys in point of direction. We read of cases where simple bottles at times of strong on-shore winds have been cast on the beach, whereas their fellow coupled bottles escaped such a fate. Still, Gilson gives no account of just how his simple surface bottles floated, and there is a special point of interest which occurs to the present writer in connection with his experiments.

If the floating buoyant upper members of his coupled systems were found stranded *minus* their drag members, it can hardly have been possible for Gilson to know whether these bottles had finished their journeys whilst still properly linked. This is a point which has greatly exercised the writer in his experiments, because it is so clearly possible to receive many records which may or may not be above suspicion. Many drag bottles may become moored close inshore whilst complete, and though the severance of the links may take place at some distance from

* In this present paper experiments with bottles entirely unballasted are described—see below, page 267.

the beach, yet the upper bottles containing the postcards may go ashore in the immediate vicinity and be perfectly reliable witnesses to acceptable travels. An attempt to get over this difficulty has been made, as will be seen later. No doubt many of Gilson's coupled systems, from the upper bottles of which he received postcards indicating that the bottle systems had become "decoupled" before discovery of the questionnaire units, were found at places to which the complete system *had* travelled, but the element of doubt cannot but have existed.

In the case of the very fine Italian experiments (**16**), the postcards definitely asked whether when the postcard bottle was found it was alone or fastened to another. Of great interest in connection with these experiments are the entries in the tabulations of returns showing that many coupled systems were recovered still coupled long before stranding, and of particular interest is it to note that some such systems were retrieved complete at distances from the beach, which can mean only that they were moored there by reason of the suspended bottles having grounded. In our rougher northern waters we are much less likely to be so favoured, but it nevertheless remains our chief aim to have drag bottles retrieved whilst still complete before their suspension cords or wires have become broken. An additional point of interest in these Italian researches lies in the fact that they evidently employed an upper questionnaire bottle almost identical with that since devised and thought novel by the present writer (see **4**, Fig. 2). This bottle was made in quantity for the writer by a commercial firm of bottle manufacturers* to permit drag bottles to be made up easily, but the Italian experimenters have the credit of having had this idea first carried into practical effect. The bottle in question has a pierced bulge at its base to permit the easy attachment of a cord or a wire.

Before passing on to consider the experiments which are the major concern of this paper one may note (**8**, p. 438) that coupled bottles were used a considerable time ago by Hautreux in the Bay of Biscay.

THE DRIFT-BOTTLE EXPERIMENTS OF AUGUST 1927, AUGUST 1928, AND JULY 1929 IN THE ENGLISH CHANNEL.

We may recall the fact that we desired to ascertain whether there would be any significant differences in the journeys performed by bottles of different types put out at such an ideal place for the purpose as E2. A very important secondary consideration was our desire to become possessed of ample good material upon which to base attempts to work out quantitative relationships between wind and bottle travel. The

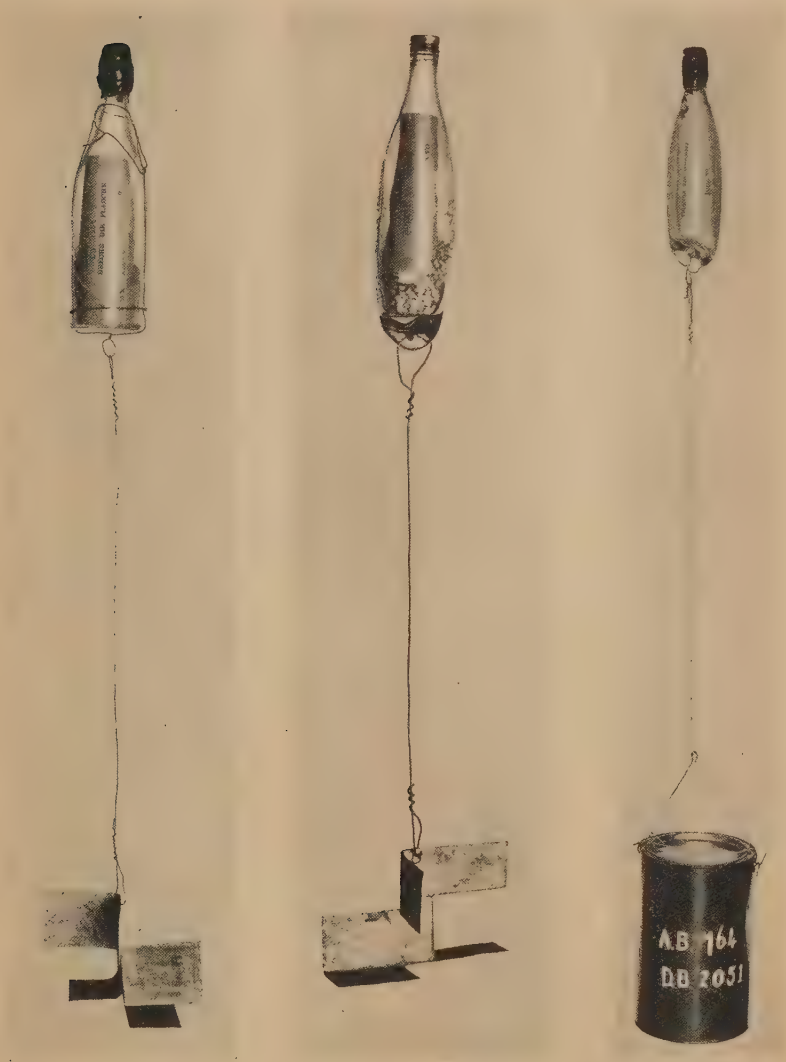
* By Messrs. Redfearn Bros. of Barnsley.

bottles were of three kinds, (1) ordinary simple surface floaters properly ballasted to float just awash, (2) bottles entirely unballasted so that wind could be expected to play its maximal rôle in influencing their movements, and (3) bottles fitted with sub-surface drags and expected to perform journeys as little affected by direct wind influence as possible.

The Bottles.

The simple surface floaters used in all three years need no comment; they are figured in (7). The completely unballasted bottles used in 1928 were what are known in the trade as "oval twelves," and they contained nothing save the questionnaire card and papers. The other bottles are figured opposite.

The drag bottles put out in August 1927 were of the type shown in Fig. 1 (a). The wire suspension was half a fathom long and the drag was that previously described (7, p. 7). The wire used had to be thinner than desirable in order that it might be manipulated satisfactorily in making the attachment. Actually, these floats closely resemble those which were used by the American investigators in their researches in the Bay of Fundy. Our bottles were of 20-oz. capacity. No means save correspondence with the finders was available to enable us to learn whether the drag was still attached when a bottle was found. These bottles were far from satisfactory, and unless we have known that the drags were still attached when found, the records have been regarded as unreliable. The drag-fitted bottles set adrift at E2 in August 1928 were of two kinds, as pictured in Fig. 1 (types b and c). Those with the metal drag were made up by using bottles of the special type already mentioned and whose unusual features can be seen in the illustration. In this case stronger wire was used, the bottles were sealed by means of screw-on copper caps, the suspension wire was 3 ft. long, and, in general, the systems were much better than those used in the previous year. The questionnaire postcards in this case definitely asked whether the drags were attached when the bottles were found. It may be seen that these bottles are easy to make up, since, when the drags are attached to the empty bottles, several dozen can be floated in a tank and sand poured in from a jug until ballasted satisfactorily. The second type used were designed to serve as drag systems, having specially large drags, the idea being to learn whether these would travel significantly differently from the bottles carrying the smaller metal drags. A number of large cylindrical brightly coloured toffee tins were obtained new (the one figured was painted for photography purposes) from the makers. These tins were nine inches high and of six inches diameter. It was thought inadvisable to ballast the tins and rely upon their proving watertight; holes were



a. b. c.

FIG. 1.—Illustrating various types of drag-fitted drift bottles.

punched in them and an unweighted bottle containing a postcard was suitably "imprisoned" in each of them before hanging the tins from the upper bottles. The underlying idea here was, of course, the thought that we might in some cases receive both postcards back from the same stranding place, which would be a very welcome experience indeed. Again, the lower buoyant bottle contained in the tin made it possible to have a larger drag than would otherwise have been practicable. The suspension wires were of good quality and were 3 feet long.

The drag bottles used in 1929 (not figured here) were better altogether, and consisted of two equal bottles linked together by means of a length (3 feet) of stout sashcord. Only the upper bottles contained postcards, and the bottom ones were so ballasted that the whole systems when afloat left only the smallest portion of the upper buoyant bottles exposed. The attachment of the cords was effected by means of loops of very stout wire fastened to the bottles by means of a device which we have found exceedingly useful, and whose nature can be seen from the pictures of Fig. 2. A monel metal cap pressed specially by the makers to bell out at the open end is screwed home on to the bottle neck to bear down upon a monel metal disc previously placed in position. The "disc" is an annulus; the central hole is just big enough to take the bottle neck, and there are holes punched in it to accommodate wires. Thus the attachments are easily made.

Being unwilling to rest satisfied until sufficient success had been achieved in these experiments as measured by the attainment of the aims stated above, one further liberation of bottles has since been made at E2 just prior to the time of writing. This time we have used material which we have good reason to hope will prove quite successful. Besides the customary simple surface floaters we have put out bottle systems of the type figured below (Fig. 2). In this case the bottle systems are much less likely to "come adrift," and it is hoped that some will be retrieved entire. It is hoped to present the results of this latest experiment next year in the form of a brief supplement to this paper. Then we shall have surface drift-bottle records from experiments in five separate years, and will find ourselves, it is hoped, in a position to speak with confidence as to the correspondence between wind and surface current in the English Channel.

The coupled bottle systems put out in July 1930, and which are much superior in strength and promise to others used earlier, are shown in Figure 2.

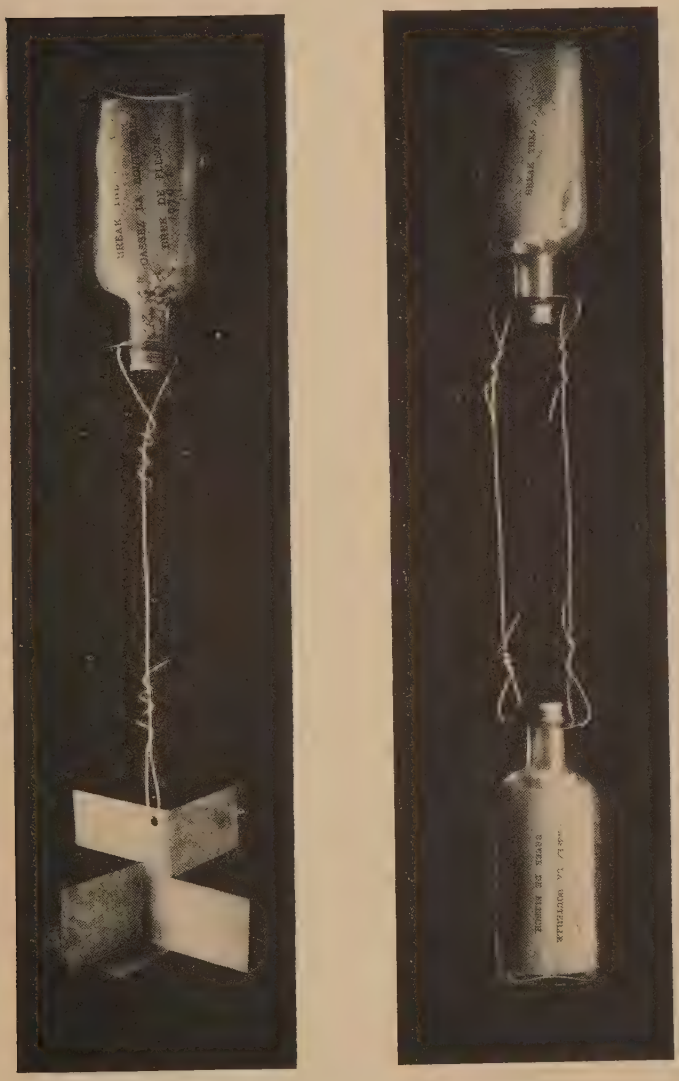


FIG. 2.—Illustrating improved types of drag-fitted drift bottles.

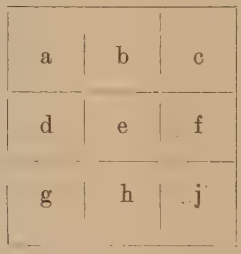
The Recoveries from the Experiments under Review.

The following procedure has again been adopted in reporting :—

A chart of sufficient geographical range was prepared embracing the whole of the English Channel, the North Sea as far north as the Shetlands, and the Cattegat—with the Belts. On this chart were superscribed the ordinary statistical rectangles, each rectangle being delimited by one degree of longitude and one-half a degree of latitude. The positions of recovery of the bottles were (as the records were received) referred to and tabulated in terms of these statistical rectangles. For greater precision in the statement of results, the rectangles were visually divided up into sub-rectangles after the manner of Army cartography practice, thus :—

the rectangle

can be considered to be subdivided thus :—



so that the position of recovery of a bottle might be tabulated as :—

G2 g,

and the latter can be much more quickly referred to a chart than could the usual co-ordinates of latitude and longitude. Chart 1 shows the position at which bottles were put out ; it is also divided up in the manner described to serve reference requirements for the recovery positions of the bottles. Remembering that all the bottles here dealt with were put out at E2, we may proceed to tabulate the details of recovery.

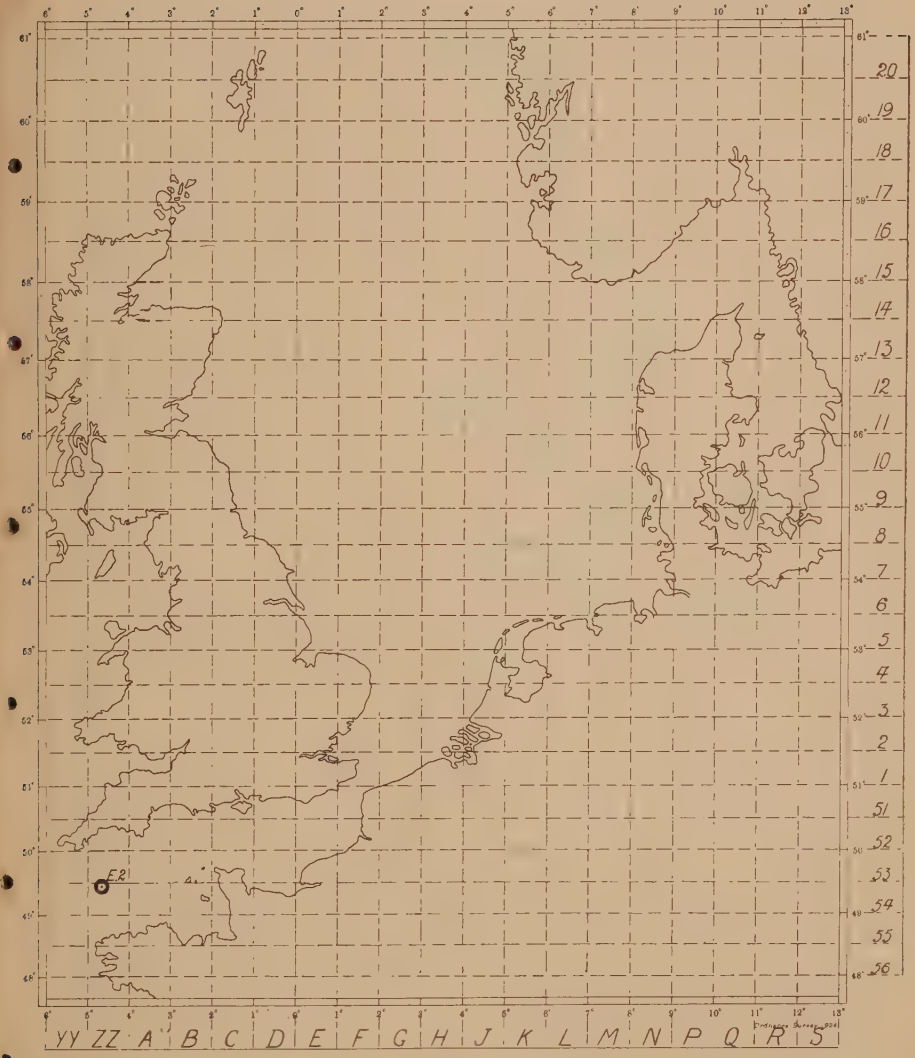


CHART 1.

Key Chart to which tabulated recovery positions of bottles may be referred.

TABLE I.

Experiment of 1927. (1) Drag Bottles (Fig. 1, a).

Liberation date : 2.8.27.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.*
12	H 2 h	79	328
14	J 5 f	97	440
14	J 4 b	97	410
14	J 4 c	97	410
21	C 9 b	146	552
22	D 6 f	148	456
22	C 8 j	149	515
27	K 16 e	189	730

Number put out 30
Number recovered 8
Percentage 26·7

TABLE II.

(2) Simple Surface Bottles.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
10	J 3 g	65	378	5·8
10	,,	66	372	5·6
13	J 4 c	86	420	4·9
13	,,	86	410	4·8
13	J 5 j	86	418	4·9
13	J 4 c	86	410	4·8
13	J 5 j	89	420	4·7
13	,,	86	420	4·9
13	J 4 c	86	410	4·8
14	K 5 a	96	450	4·7
14	N 8 a	97	585	6·0
31	K 16 e	201	730	3·6
37	N 26 g	257	1290	5·0
51	S 31 j	353	1250	3·5

Number put out 25
Number recovered 14
Percentage 56·0

* Lengths of the shortest tracks which can be drawn on the chart without crossing land. These distances are in many cases certainly much too small—particularly where bottles recovered beyond the Skaggerak are concerned.

TABLE III.

Experiment of 1928. (1) Completely Unballasted Bottles.

Liberation date : 10.8.28.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
4	D 51 d	27	167	6·2
4	C 51 h	28	147	5·3
4	C 51 j	28	153	5·5
4	,,	28	153	5·5
4	,,	28	155	5·5
4	,,	28	155	5·5
4	,,	28	153	5·5
4	,,	28	153	5·5
4	C 51 e	28	146	5·2
5	C 51 f	29	158	5·5
5	,,	31	162	5·2
5	,,	34	159	4·7
5	,,	34	161	4·7
5	C 51 j	34	155	4·6
7	C 53 f	45	134	3·0
7	C 54 c	48	155	3·2
7	C 53 j	48	145	3·0
11	E 51 c	71	230	3·2
12	E 51 d	78	202	2·6

Number put out	25
Number recovered	19
Percentage	76·0

TABLE IV.

(2) Simple Surface Bottles.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
11	D 51 d	71	167	2·4
11	C 51 g	72	130	1·8
11	C 51 h	72	147	2·0
11	,,	73	146	2·0
11	,,	74	147	2·0
11	D 51 c	75	192	2·6
11	C 51 j	75	153	2·0
11	D 51 c	77	192	2·5

TABLE IV—*continued.*

Weeks from liberation.	Position of recovery (refer to Chart I).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
11	D 51 d	77	173	2·3
11	,,	77	172	2·2
12	C 51 b	78	155	2·0
12	E 51 b	78	220	2·8
12	D 51 e	78	177	2·3
12	,,	78	177	2·3
12	,,	78	187	2·4

Number put out	75
Number recovered	15
Percentage	20·0

TABLE V.

(3) Drag Bottles. (Fig 1 b.)

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
12	D 51 c	78	192	2·5
12	E 51 d	78	198	2·5
15	,,	102	200	2·0
16	C 54 a	108	116	1·1
16	H 2 h	111	338	3·0
16	L 6 j	111	505	4·5
23	H 1 d	161	310	1·9
37	J 5 j	259	420	1·6
38	J 4 c	260	418	1·6
46	L 6 j	322	510	1·6
47	N 10 a	325	625	1·9
47	M 6 d	325	540	1·7
48	N 6 b	333	577	1·7
48	N 7 j	333	586	1·8
48	,,	334	586	1·8
48	N 7 e	336	582	1·7
49	P 6 a	342	600	1·8
51	N 7 j	351	685	2·0
51	N 9 f	351	618	1·8
71	N 6 c	491	575	1·2

Number put out	50
Number recovered	20
Percentage	40

Table VI.

(4) "Toffee-Tin" Drag Bottles. (Fig. 1, c.)

(SYSTEMS BOTH OF WHOSE BOTTLES WERE RECOVERED).

Bottle concerned.	Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
Upper	11	ZZ 2 e	71	170	2·4*
Lower	11	D 51 c	76	192	2·5
Upper	11	B 51 h	74	110	1·5*
Lower	11	C 51 e	71	143	2·0
Upper	11	D 51 d	76	168	2·2*
Lower	11	D 51 e	74	180	2·4
Upper	11	D 51 c	77	186	2·4
Lower	16	F 51 e	107	256	2·4

NOTE.—The "lower" bottles, i.e. those initially fastened in the tins, could only themselves perform journeys on escaping from their tins; in this eventuality the upper bottles would sink so long as wire and tin remained hanging from them.

(SINGLE RECOVERIES).

Member.	Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
Lower	11	D 51 e	76	180	2·4
Upper	11	D 51 c	76	196	2·6*
Upper	11	C 51 h	72	147	2·0
Upper	11	D 51 e	74	182	2·5*
Lower	11	D 51 c	74	186	2·5
Upper	15	E 51 d	105	200	1·9
Lower	11	E 51 b	75	220	2·9
Upper	11	B5 1 j	71	102	1·4*
Lower	10	C 51 g	64	130	2·0
Upper	11	D 51 c	75	190	2·5*

Number of pairs put out—25.

Number of pairs recovered intact—Nil.

Number of pairs both of whose bottles were recovered—4.

Number of single bottles recovered—10.

Number of bottles recovered in all—18.

* Denotes stated absence of attachment when found.

Table VII.

Experiment of 1929. (1) Sashcord Linked Coupled Bottles.

Liberation date : 30.7.29.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
11	C 54 a	72	115	1·6
37	F 52 a	253	228	0·9

Number put out 47
Number recovered 2

Note.—In this case there was only one postcard, which was in the lower non-buoyant suspended bottle. Thus any recovery is witness of a journey performed with the system intact. The fewness of the returns may be due to breakage of the suspension cords ; if so (1) we are well rid of the useless postcards which we should have recovered had we put cards also in the upper bottles, and (2) we may perhaps later receive some postcards from bottles retrieved by trawl.

Table VIII.

(2) Simple Surface Bottles.

Weeks from liberation.	Position of recovery (refer to Chart 1).	No. of days "out."	Distance travelled in miles.	Corresponding speed in miles per day.
6	B 55 g	38	102	2·7
6	B 55 d	40	82	2·1
7	B 55 h	46	102	2·2
7	B 55 j	48	114	2·4
8	B 55 j	50	108	2·2
8	B 55 d	50	88	1·8
9	,,	60	82	1·4
10	E 51 c	70	220	3·1
11	C 54 a	71	114	1·6
11	,,	71	114	1·6
11	F 51 e	73	252	3·5
11	C 54 a	74	115	1·6
11	C 54 h	77	126	1·6
12	C 54 d	82	118	1·4
13	C 54 a	91	114	1·3
14	F 51 c	93	259	2·8
20	N 10 g	138	620	4·5
20	P 14 j	139	740	5·3
22	N 10 a	148	625	4·2
24	Q 17 e	166	850	5·1

Number put out 39
Number recovered 20
Percentage 51·3

GENERAL COMMENTS.

The extent to which the records from the various types of drag bottles used in the three experiments are to be regarded as reliable will be indicated in the discussion which follows. It is clear that we must set down some account of the relevant wind conditions, and it would be a great convenience if some of the more interesting bottle journeys were represented pictorially, as was done by means of charts in the earlier paper in this Journal (**3**). However, it is not possible here to present charts except for the more interesting records.

To present for each year detailed wind data from various suitable observing stations would occupy too much space. Such data as do demand inclusion must yield the following: (1) Information as to wind at some place not too far from E2, at least for a period just after the bottle liberation in that year which saw the putting out of the completely unballasted bottles, and (2) the most acceptable information for each year concerning what may be regarded as the average wind conditions applicable to the entire journeys of those simple surface bottles which moved right up-Channel into the North Sea. In this latter connection it might readily be agreed that suitable continuous wind records made at some place well up-Channel from E2 would best serve; in the considered opinion of the writer the most useful data for the purposes in view are the autographic wind data from the Eastbourne Observing Authority. We mean that these data are considered likely to reflect the mean wind conditions over the track of the far-travelled bottles as well as any we could turn to, and they have the merit of being very convenient.

It is deemed very desirable to seek some quantitative relationship between surface current and wind, a problem which can only be viewed as a series of steps. The fullest and most reliable bottle-travel data at present available are the travels of the simple surface floaters, since with these one pays attention to the travels of the fastest long-distance individuals—a convenience not possible in the case of the drag bottles for obvious reasons. It seems perfectly reasonable, therefore, to attempt first to associate wind and *surface* bottle journeys, hoping later to deduce some amending factor from results obtained with drag-fitted bottles, and to apply this amending factor later to whatever inter-relationship we may have been able to establish between wind and surface-bottle travel. This would be a step nearer to satisfaction, considering that surface current is what we ultimately wish to deal with.

As regards wind in this connection, it seems clear that we can best concern ourselves with data setting forth the daily mileage run of the wind up-Channel (as deduced from Eastbourne autographic records) for periods of time covering the journeys of the long-distance bottles. This

means that, if we regard the trend of the Channel as S.W. to N.E., we must learn what run of N.E. going wind has been associated with the bottle journeys. In obtaining our wind data we should in this case simply regard S.W. going wind as of negative sign, knowing that over several months it cannot predominate. One feels that the numerical association of long period means of up-Channel winds with long bottle travels, is the only possible procedure in attempting to work out any useful quantitative relationship between bottle travel and wind, when dealing with an area like the Channel whose waters are always in tidal motion. The phrasing here intentionally avoids the implication that bottle travel and surface current are one and the same thing. With the remark that the wind data applicable to the 1924 bottle experiments from E2 were very simply handled, since the wind was uninterruptedly "up-Channel," we may pass on to set down such data as suit our present study.

Table IX.

RELEVANT WIND DATA FROM EASTBOURNE AUTOGRAPHIC RECORDS.

[Extracted from the appropriate yearly Meteorological Reports of the Eastbourne County Borough and thrown into a form suited to the present study. By "Up-Channel winds" we mean SW.+W.+NW. and by "Down-Channel winds" NE.+E.+SE.]

Period.	Total miles run by wind.	Daily mean mileage run of wind.	Corresponding hourly mean.	Derived multiplying factor to obtain up-Channel wind.	Derived multiplying factor to obtain down-Channel wind.
Aug. 1927	6076·00	196·00	8·17	0·69	0·20
Sept. ,,	6420·60	214·02	8·92	0·77	0·10
Oct. ,,	5567·12	179·58	7·48	0·62	0·15
Above Period	18063·72	196·35	8·18	0·69	0·15
Aug. 1928	5811·04	187·45	7·81	0·69	0·15
Sept. ,,	4450·48	148·30	6·18	0·38	0·33
Above Period	10261·52	168·22	7·01	0·53	0·24
Aug. 1929	5178·40	167·05	6·96	0·72	0·17
Sept. ,,	4088·90	136·30	5·68	0·49	0·36
Oct. ,,	6845·40	220·82	9·20	0·77	0·06
Nov. ,,	6998·28	233·28	9·72	0·64	0·06
Above Period	23110·98	189·43	7·89	0·67	0·16

DAILY MILEAGE RUN OF "UP-CHANNEL" AND "DOWN-CHANNEL" WIND AS DERIVED FROM THE FOREGOING TABLE.

Period.	up-Channel.	down-Channel.	Balance up-Channel.
Aug. 1927	135·24	39·20	96·04
Sept. ,,	164·80	21·40	143·40
Oct. ,,	111·34	26·94	84·40
Foregoing Three Months	135·48	29·45	106·03
Aug. 1928	129·34	28·12	101·22
Sept. ,,	56·35	48·94	7·41
Foregoing Two Months	89·16	40·37	48·79
Aug. 1929	120·28	28·40	91·88
Sept. ,,	66·79	49·07	17·72
Oct. ,,	170·03	13·25	156·78
Nov. ,,	149·30	14·00	135·30
Foregoing Four Months	126·92	30·31	96·61

[On treating the corresponding wind data for the three months July, August, and September of 1924 by this procedure, we obtain the figure 131·06 as the residual "up Channel" daily run of the wind in miles.]

Additional information which may be set down as likely to be definitely illuminating, concerns the known flow of water through Dover Straits at times germane to our present study. Long-period averages only are what we want in this connection, and such Dover Straits records are in the writer's hands in the form necessary.*

As a glance at the charts (Charts 2, 3 and 4) or at the tabulations of bottle recoveries shows, there must have been considerable differences between the three experiments as regards the paths of the bottles in the earlier stages of their wanderings. It is readily surmised that this may have been due to differing wind conditions in the western Channel, and it is necessary to see if such differences did characterise the months concerned in each case. If one can establish such an explanation something of importance will have been accomplished, for we know far too little of the influence of wind on water movement in the area concerned. One experiment would be of little avail in this connection, but we have four experiments (counting that of 1924 already published) in four different summers, and since, as is clear, the bottles moved off differently

* We refer to the continuous current measurements in train at the Varne Lightvessel.

our data can be regarded as being of special promise. We can get the requisite information respecting appropriate wind conditions from the relevant annual "Meteorological Notes" of Falmouth Observatory. Suitable treatment of the published data yields the following information:—

Table X.

WIND DATA FROM FALMOUTH

Period.	Direction and duration of residual wind.
Aug. 1927	360 hours from S. 59° W. true.
Sept. ,,	420 ,, N. 70° W. ,,
Aug. 1928	370 ,, S. 57° W. ,,
Sept. ,,	86 ,, due E. ,,
Aug. 1929	425 ,, S. 86° W. ,,
Sept. ,,	145 ,, N. 12° W. ,,

Before proceeding to discuss the bottle experiments we will present that remaining set of data which might be expected to bear upon our study—the available information as to the contemporaneous flow of water through Dover Straits.

Table XI.

THE FLOW OF WATER THROUGH DOVER STRAITS AT TIMES CONCERNED

Period.	Mean daily flow in miles per lunar day.
Aug. 1927	3·21 towards N. 25° E. true.
Sept. ,,	3·47 ,, N. 32° E. ,,
Oct. ,,	3·34 ,, N. 40° E. ,,
Aug. 1928	3·20 ,, N. 36° E. ,,
Sept. ,,	1·30 ,, N. 7° E. ,,
Oct. ,,	5·80 ,, N. 17° E. ,,
Aug. 1929	3·80 ,, N. 22° E. ,,
Sept. ,,	1·80 ,, N. 12° W. ,,
Oct. ,,	4·10 ,, N. 25° E. ,,
Nov. ,,	7·10 ,, N. 46° E. ,,
Dec. ,,	11·40 ,, N. 49° E. ,,

DISCUSSION OF THE EXPERIMENTS.

It will be convenient first to comment upon the individual experiments in turn, and later to make comparisons and draw contrasts between them.

Experiment of 2nd August, 1927.

In Table I have been set down the histories of the drag bottles put out. These bottles (see Fig. 1, a) were, as experience has shown, far from good enough. The wire was, of necessity, much thinner than desirable, and it is a safe inference to make that many of the suspension wires must have broken. It is fruitless to discuss these drag bottles in any detail, and one is not prepared to place any real reliance upon the apparent speeds attained by those individuals which stranded on the continental coast after 12 to 14 weeks. There is ample reason for this reluctance. Although the bottles which did so strand exhibit no excessive speed as compared with their fellow simple surface floaters, yet a consideration does arise which makes them suspect. One bottle was found on the Yorkshire coast, another near the Tyne, and one further north. This is indeed surprising, and can only mean that these bottles lost their drags, became much under wind influence, and were literally blown across the North Sea by the strong S.E. winds of December 1927.* It may be that some of the bottles which stranded earlier on the continental coasts had also lost their drags but had gone ashore before the onset of the December easterly winds, which caught the others still afloat. However that may be, here we have strong evidence of the potent influence of winds in influencing the travel of partially submerged bottles, and in causing them to perform journeys which properly ballasted bottles were not constrained to do.

As regards the simple surface floaters (see Table II), none stranded short of the North Sea which, in view of the winds tabulated above, is not surprising. The tracks can be seen in Chart 2, and that journey of 585 miles in 97 days will be referred to again. We may assume a speed of travel up-Channel on the part of these bottles of quite 6 miles a day.

Experiment of 10th August, 1928.

In this case no simple surface floater (see Table IV) was recovered beyond Kent ; all the recoveries were made on the south coast of England along a stretch between St. Alban's Head and Pevensey. Probably the overall rate of travel of the fastest was about 3 miles a day (see Chart 3). The easterly winds of September 1928 are to be held accountable for these facts.

* The residual wind for Gorleston over the period of 5 weeks commencing 20th November, 1927, was : 4·6 m.p.h. from S. 70° E. true.

CHART 2.

Illustrating the journeys accomplished by simple surface floating bottles put out at International Station E2 on 2nd August, 1927. Where the arrows are multi-barbed, the number of barbs denotes the number of bottles recovered at the place indicated. The figures in the circles refer to the stranding places near by, and have the following signification :—

The numerator indicates chordal distance travelled in miles.
The denominator gives the time " out " (in days) of the fastest bottle.
The bracketed figures denote the corresponding speed in miles per day.

The completely unballasted bottles (see Table III) were most obviously at the mercy of the strong south-westerly winds which carried them on to the English coast around the Isle of Wight. They covered the short distance of some 150 miles at a speed of about 6 miles a day. Three individuals evidently came offshore again to strand later on the east coast of the Manche département—a fact which a study of the winds already presented can elucidate. It is of very great interest indeed to note that none of the properly ballasted surface floaters accompanied them to the

CHART 3.

Illustrating the journeys accomplished by simple surface floating bottles set adrift at International Station E2 on 10th August, 1928.

The figures in circles refer to the strandings near by, and have the same signification as on Chart 2—as also have the arrows.

Manche coast. Here then is an answer to those workers who have not thought it essential to ballast their drift bottles with great care.

The drag bottles put out (see Fig. 1, b) were of a type in which considerable reliance could be placed. A glance at Table V reveals much of interest. The journeys accomplished were very significantly different from those performed by both simple surface floaters and completely unballasted bottles. Recoveries were made all along the continental coasts from Belgium to north Jutland (several being recovered right in the German Bight), whereas no simple surface floater was recovered clear of the English

Channel. Why was this ? Only four of these drag bottle returns came from the English Channel, and it cannot be denied that these strandings may have been consequent upon loss of the drag. The balance of probability is that such was the case.

Here surely is indisputable evidence that the travels performed by drag-fitted bottles may be markedly different from those of simple surface floaters put out at the same time and place. We see clearly that the wind was unable to influence the movements of these drag-fitted bottles to nearly the same degree as those of their fellow simple floaters. We could not have wished wind conditions to have been other than what they were, seeing what striking results we have obtained, but at least they have deprived us of one piece of information which would have been very welcome. We had hoped from this experiment, to have learnt by what amount (if any) the speed of drag-fitted bottles falls in defect of that of simple surface floaters put out at the same time and place, for, as has been said earlier, we had wished to be able to apply an " amending factor " to the quantitative relationship to be presented connecting wind with travel of simple surface floaters. The idea here was, of course, to get information more acceptable as representing surface drift, than does perhaps the travel of simple surface bottles. Such information as was wished for, cannot be gleaned from the experiments in 1928, since the two types of bottle did not perform comparable journeys. One may note in passing, that the predominant speed of these drag bottles was round about $1\frac{1}{2}$ to 2 miles a day.

It remains to comment on our experiences with the bottles like that shown above (see Fig. 1, c). These " toffee-tin " bottles, as we may conveniently call them, cannot be considered to have proved much of a success. Not one system was recovered intact (see Table VI), but there emerge a few facts of interest. It is highly probable that the two bottles (both members of the same pair) which had stranded in the rectangle D51 did journey, there in concert, and we note that the speed was about 2·4 miles a day. Those bottles which were originally " imprisoned " in the tins, must, for some reason, have got free, perhaps owing to the tins perishing. Only by getting free could they avoid being carried to the bottom on the rupture of the suspending wires. One upper bottle on losing its large drag, journeyed to South Wales. It is to be noted that not one bottle (neither upper nor lower member) got free of the English Channel, the majority stranding on the south coast of England east of 3° W. longitude. This is very illuminating in view of what has gone before. Had they proceeded on and on as perfect drag bottles, we should (on the analogy of the results from the other drag bottles put out with them) have expected them to have got clear into the North Sea. Obviously they have proved to be " neither fish, fowl nor good red herring," though they have not been entirely

without interest. It is probable that these "toffee tin" systems broke up just as the easterly winds which so affected the movements of the simple surface floaters, commenced.

Experiment of 30th July, 1929.

Much was hoped for from the coupled-bottle systems put out, but as yet (see Table VII) only two returns are to hand. This leaves us completely unable to assess speed satisfactorily; for this purpose we require a number of returns to get rid of the difficulties arising out of the possibility that the bottles may have lain undiscovered for a considerable time. As regards direction of travel, the meagre records are quite good, for the postcard bottles can only have performed journeys at all so long as they hung from their buoyant partners. One suspects that many of these postcard bottles are lying at the bottom of the sea somewhere in very shallow waters just off-shore. The upper bottles were purposely devoid of postcards. Actually the further travelled bottle was recovered by trawl 29 miles north of Dieppe. The stranding place of the less travelled of the two bottles recovered is of interest in the light of the frequent northerly winds of September. As will be noted later, the simple surface-floating bottles were carried into the Bay of Saint Malo, and this drag bottle must also have been urged thither by the wind, for it failed to round Cap de la Hague. This fact speaks much for the influence of the wind on this occasion.

The journeys performed by the simple surface floaters put out on 30th July, 1929, are very striking (see Chart 4 and Table VIII). They were carried first into the Bay of Saint Malo and stranded round its shores after travelling at a speed low enough to make one surmise that they had been, as it were, hovering about in the open sea for some time under wind conditions not making for quick travel. With the onset of the frequent northerly winds of September they were evidently urged ashore. Such individuals as succeeded in rounding Cap de la Hague went on up-Channel, and some of them reached the Skagerrak.

Brief Review of the Main Results of the Three Experiments.

Common surface-floating bottles put out at the same place (E2) in the same part of the year in 1927, 1928 and 1929 have exhibited very marked differences in their travels; these differences have been seen to reflect the wind conditions which characterised the three occasions.

In one of the experiments (liberation of 10.viii.1928) completely unballasted bottles, common surface-floating bottles properly ballasted, and drag-fitted bottles of two kinds were put out at the same time and

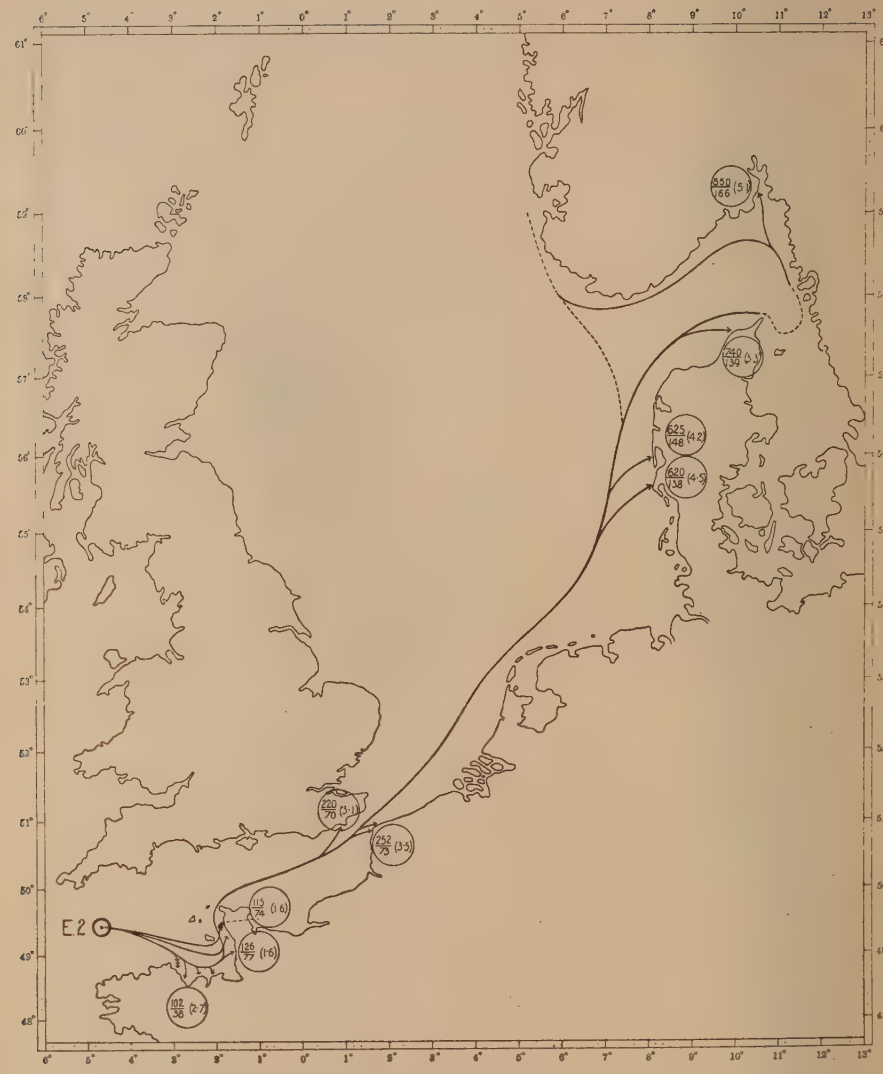


CHART 4.

Illustrating the journeys accomplished by simple surface floating bottles set adrift at International Station E2 on 30th July, 1929.

The figures in circles refer to the strandings near by, and have the same signification as on Chart 2—as also have the arrows.

place. The travels of the different types have been seen to have differed very significantly. The associated wind conditions were, of course, the same for all four kinds of bottle, but the influence which the wind-urged surface water had on the different types in determining their travels has proved strikingly variant.

In the case of the 1927 experiment, when the bottles were carried straight up-Channel without any stranding short of the Dutch islands, there was a continuous flow of water through the Straits of Dover of about $3\frac{1}{3}$ miles a day (see Table XI) during each of the three ensuing months.

Whilst August 1928 differed hardly at all from August 1927 in point of water flow through Dover Straits, yet September 1928 was a month characterised by strong reversals of the usual E.N.E. going flow from English Channel to North Sea. This fact throws much light on the travels of the 1928 simple surface-floating bottles. August 1928 must have been a month of good travel up-Channel, but in September 1928 the bottles were constrained to strand on the south coast of England. The extra brisk flow of water through the Straits in October 1928 was too late to give rise to subsequent strandings on the North Sea shores.

August 1929 was, so far as the Dover Straits current is concerned, little different from the same month in the two previous years, but September 1929 was quite remarkable. In this month the flow of water from English Channel to North Sea was completely held up ; the resulting condition in the Channel must have been that no pronounced drift of water existed. Thus local winds could play a specially strong rôle, and we have seen how the 1929 surface bottles, after drifting about the Bay of Saint Malo for some time, made progress up-Channel, and how three individuals stranded on the coasts bordering Dover Straits. The latter fact is of special interest in view of the contemporaneous hold-up of the water flow through the Straits.

Thereafter, in 1929, the Dover Straits flow of water waxed so very greatly in strength that bottles which had escaped stranding were able to accomplish such rapid journeys across the North Sea that their overall speed for their complete journey touches the high figure of at least 5 miles a day.

We have now one remaining task—to see if we can find any relationship of a numerical kind between the two quantities :—

(1) Speed of Surface-Bottle Travel, and
(2) Speed of Wind at the time.

It is necessary to emphasise here that one must just apply ordinary common sense to the problem. We are not dealing with quantities of a rigid character ; what we must use if we can get them are values for

bottle travel and wind, which are acceptable as being the most comparable we could arrive at. The waters of the English Channel being continually in movement as a result of the tidal streams, we have no simple case of wind action on a placid body of water to tackle. It is necessary to proceed as follows :—

Suppose that bottles have moved up-Channel from E2 and have accomplished journeys of several hundred miles. We must, for our purpose, get ample wind data (preferably autographic) from some place situated, say, half-way along the bottle tracks, and work out the appropriate residual wind over the period of time occupied by the bottles' journeys.

It is difficult to see how one can allow sufficiently for the tidal streams if one seeks to establish the desired relationship any other way. Even if we do arrive at an acceptable result, we may not necessarily assume it to be applicable to other waters than those with which we are here concerned.

Over a period of time exceeding a few months, the balance of wind direction will always be up-Channel. We have earlier set down the requisite wind data for our present purpose (see Table IX), and they, together with the bottle-travel data with which we may associate them, will here be given. It is to be noted that the bottle data refer to the simple surface bottles for each year, and attention is drawn to the fact that we are not begging the question by tacitly assuming that bottle travel and surface current are the same thing. They may be so within a little, but we must wait until we have ample satisfactory contemporaneous data from drag bottles and simple surface bottles to work out what we have earlier called an " amending factor." In a previous paper (3) the writer was merely able to *fit* a constant to a type of wind-bottle equation. We are now able to go much further and to establish an equation for our area. We have re-cast the relevant data from our 1924 experiments for inclusion here, and in all have data from four different experiments at our disposal. Here are the quantities which call for our attention :—

Experiment.	Mean daily travel of fastest long-distance bottle* (miles per day).	Mileage run of " up-Channel " wind per day during relevant period.
July, 1924	8	130
August, 1927	6	105
August, 1928	2·8	50
July, 1929	5·3	95

* Having regard only to bottles which did not go beyond the Skagerrak ; beyond this the distances actually covered can only be very roughly assessed.

It would indeed be foolish, considering the kind of data with which we are dealing, to seek to deduce any equation other than a simple one. The inter-relationship between the quantities just set down can be expressed by the following simple equation as satisfactorily as by any other :—

$$S = \tfrac{1}{18} W.$$

where S is bottle travel in miles per day and W the wind speed in the same units—both being worked out over considerable periods as herein done.

ADDENDUM.

Since this paper was written, it has been felt that fuller wind data from the western English Channel could, with advantage, have been included. Accordingly, additional suitable records have since been worked up into the form of residual winds—this being a conveniently brief way of presenting much information in a small space. It was thought that by far the best thing to do would be to extract from the relevant daily weather reports, the records published for Scilly and Guernsey (four observations daily in each case) and to add these together so as to obtain results particularly representative of the average wind conditions over the western Channel. The following table presents the resulting information :—

Residual Wind Data relating to Scilly and Guernsey considered as one Observing Station.

1927	August	9·3	m.p.h. from	S.67° W. true.
	September	9·1	„ „	N.88° W. „
1928	August	7·0	m.p.h. from	S.63° W. true.
	September	3·7	„ „	N.75° E. „
1929	August	7·1	m.p.h. from	due W. true.
	September	2·0	„ „	N.19° E. „

The fact that the simple surface bottles of 1927 travelled rapidly up-Channel and into the North Sea, is not surprising in view of the wind data just set down. In that year, the winds were overwhelmingly more favourable to the accomplishment of up-Channel travel than they proved to be in 1928, and much more favourable than those which prevailed in 1929.

All the bottles put out in 1928 must have set off up-Channel at a good speed aided by the favouring winds of August. The unballasted bottles reached the longitude of the Isle of Wight very quickly, being blown ashore near there within the month. The ordinary surface bottles escaped being blown ashore with their unballasted fellows, and went on up-Channel, until, before they reached the North Sea, easterly winds held up the Dover Straits current and caused them to strand on our south coast. It is to be noted that these surface bottles were not constrained to strand down wind at the time, as were the few unballasted bottles then afloat.

The extra "west in the wind" in August 1929 as compared with August 1928, made itself strongly felt. The surface bottles were carried into the Bay of Saint Malo, from which area the subsequent N.N.E.'ly winds prevented them easily escaping to travel on up-Channel.

Acknowledgment.

I am indebted to my colleague Mr. H. Stokes for preparing the fair copies of the charts from my rough drafts. The paper has been checked by the Statistical Branch of the Fisheries Department.

Many of the views elaborated in this paper have been previously dealt with in considerable detail by Professor Gilson in the course of his reviews of earlier papers by the present writer (see *Journal du Conseil*, February 1926, March 1927, and September 1928).

SUMMARY.

In July–August of three different years common surface-floating bottles were set adrift at International Station E2 (49° 27′ N. 4° 42′ W.). With them, various types of drag-fitted bottles were also put out. The journeys accomplished are discussed, and the striking differences as between year and year in the case of the common surface floaters, and as between the different types in the same year, are commented upon in the light of the prevailing winds. An inter-relationship of great simplicity is deduced between wind speed and the rate of travel of simple surface floating bottles up-Channel and across the North Sea from the results of experiments carried out in four different summers.

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Notes on the Biology of Certain Lamellibranchs on the Scottish Coast.

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With 15 Figures in the Text.

CONTENTS.

	PAGE
1. Introduction	277
2. Cardium edule	278
(*a*) Introductory	278
(*b*) Time of Spat-fall	279
(*c*) Disturbance rings	282
(*d*) Age-composition of Cardium on the Hunterston Sands	283
(*e*) Size at each winter ring. 1930 spat, Stns. 8, 13, 18, 21	284
1929 ,,	284
1928 ,,	285
1927 ,,	285
1926 ,,	287
Spat of 1925 and previous years	288
Kilchattan Bay, Bute	289
(*f*) Differences in the rate of growth between H.W.M. and L.W.M.	290
3. Tellina tenuis	291
(*a*) Variations in quantity of spat and in total population	291
(*b*) Variation in density of population	293
(*c*) Rise and decline of the 1926 spat	294
4. Macoma baltica	296
Rate of growth and time of spat-fall	296
5. Summary	298
6. Literature cited	299

1. Introduction.

Casual observations on Cardium at Millport not agreeing closely with Orton's account of his work at Plymouth (2), it was felt that there might be important differences between the two areas which would make an investigation in the Millport area of interest and importance. Further, Millport is very favourably situated for such observations since the rich,

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extensive and little disturbed cockle-beds on the Hunterston Sands are of easy access from the Laboratory. The growth and spatting of the species have been studied for a year, from the autumn of 1929 until the autumn of 1930. The results so far obtained seem of sufficient importance to warrant a preliminary account, and it is hoped later to deal with several other points in the biology of Cardium which at present are not sufficiently clear.

The observations on *Tellina tenuis* at Stn. 5 in Kames Bay, Cumbrae, are summarised.

An attempt has also been made to carry out similar work with *Macoma baltica* in the Firth of Forth. It is unfortunate that this species is not present in the necessary quantity at Millport to give results comparable with those for Cardium and Tellina.

The methods used throughout have been those originally adopted for studying *Tellina tenuis*. Unless otherwise stated collections have been made with a 2-mm. sieve, and from an area of $\frac{1}{4}$ sq. m. dug to a depth of 15 cm. The " length " of the shell, or annual ring, has been taken as the measurement in a straight line of the greatest antero-posterior diameter. All measurements have been taken to the nearest mm. above, e.g. all specimens from 4·1 to 5·0 mm. have been counted as 5 mm. The total length of the shell has been measured on a measuring board, and the measurements of the annual rings have been made with a pair of fine dividers.

For part of the material and for the facilities which I have enjoyed at the Millport Marine Station, I am indebted to the superintendent, Mr. Elmhirst, without whose ungrudging assistance the work could not have been accomplished.

2. Cardium edule.

(a) *Introductory.*

Johnstone (1) states that *Cardium edule* breeds during spring and summer, this conclusion having evidently been reached from an examination of the reproductive organs. As we now know, however, this is not always a safe guide since many species carry apparently ripe reproductive products for a considerable time before spawning. Orton (2) largely accepts his conclusion and considers that all specimens without a well-defined ring on the shell are spatted in the same year. He also concludes that the first of the several conspicuous rings usually seen on a large shell represents the *first* winter ring. While this may be the case at Plymouth it is not borne out by the observations at Millport. As will be shown later, however, the evidence on which Orton based his conclusions is open to an alternative interpretation. The conclusions reached regarding the time of spatting at Millport have been drawn, not from the doubtful

evidence of the state of the reproductive organs, but from the time of appearance of very small cockles at various stations. The evidence drawn from the observations made at Millport during 1929 and 1930 seems to point conclusively to (*a*) a summer breeding period and (*b*) to the ring laid down in the first winter being faint and easily overlooked, the first conspicuous ring being the *second*, not the *first*, winter ring.

The observations were carried out on the Hunterston Sands, which lie on the Ayrshire coast opposite the Cumbrae, and have a length of about three miles and a maximum width of about a mile at low tide. Previous to the main experiment a general survey was undertaken in the autumn of 1929 during which 26 stations, equally spaced over the sands, were examined. At each station the sand, or muddy sand, was dug out to a depth of 15 cm. from an area of $\frac{1}{4}$ sq. m. and run through a 1-mm. sieve. From the results of this survey four stations, representing the chief types of the Cardium ground, were selected for further work. These stations were as follows :—

Stn. 8. North-east part of the sands, about half-tide, in the middle of a rich cockle bed.

Stn. 13. Near H.W.M. at the upper edge of this bed close to the Black Rocks.

Stn. 18. In the middle of the sands north of Poteath, about half-tide, where the Tellina and Cardium grounds overlap.

Stn. 21. South-west end of the sands about half-tide, in the middle of a rich cockle bed.

(*b*) TIME OF SPAT-FALL.

Of the four stations No. 21 was most intensively worked and may conveniently be discussed separately.

TABLE I.*

TABLE SHOWING THE RATE OF GROWTH OF THE 1929 SPAT DURING 1930, AND THE TIME OF APPEARANCE OF THE 1930 SPAT AT STN. 21.

	Size in mm.																		Aver. size 1929 spat.	Aver. size 1930 spat.
Date.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.		
13.9.29	1	7	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4·4	.
18.4.30	2	17	9	6	2	2	1	1	.	.	.	.	.	.	.	.	.	.	5·1	.
1.7.30	.	.	.	.	.	3	6	13	20	19	16	8	5	5	2	1	.	.	12·1	.
24.7.30	.	.	.	1	.	.	3	1	2	2	5	5	5	2	1	.	1	.	13·1	.
6.8.30	28	4	.	.	.	.	.	.	.	.	1	2	1	7	1	1	1	.	15·9	3·1
4.10.30	18	28	21	6	1	1	.	.	.	.	.	.	.	.	1	1	1	.	18·0	4·3

* Since the older individuals do not come under review in this paragraph, the table is confined to specimens under 21 mm. in length, the rest of the collections being dealt with later.

In September, 1929, a group of very small cockles, ranging from 3 to 6 m.m., was found, but from 7 to 21 mm. there was a hiatus. Growth starts early in spring, but collecting was delayed until April so that the new year's growth should have had time to show, and the nature of the winter ring laid down by these fragile spat could be seen. In passing it may be remarked that in most of the specimens this ring was narrow and insignificant, but the original small shell could usually be distinguished by its darker colour.

In the April collection, while some shells had reached a length of 10 mm., the majority were still from 4 to 6 mm. in length, with an average size of 5·1 mm.; that is practically the same as in autumn. From April onwards growth was rapid, and in the beginning of July the young cockles ranged from 8 to 18 mm. with an average size of 12·1 mm. No specimens under 7 mm. were taken. Three weeks later the range was from 9 to 19 mm., with the exception of one specimen at 6 mm., and the average size was 13·1 mm.

By the beginning of August a great change had taken pace. The spat of 1929 now ranged from 13 to 19 mm. with an average size of 15·9 mm.; and a new group of small individuals ranging up to 4 mm. in length - the 1930 spat—had appeared. The figures, so far, all refer to the collections made with the 2-mm. sieve, but, in addition small samples of sand were run through a 1-mm. sieve as a check on new spat. Until August this procedure had shown that all the young cockles were being retained by the 2-mm. sieve, but in August it was evident that the numbers of cockles retained by the 2-mm. sieve represented only a small fraction of the spat, since from an area of $\frac{1}{4}$ sq. m. 51 cockles passed the 2-mm. sieve and were retained by the 1-mm. sieve.

By the beginning of October the 1929 spat was very much reduced in numbers, and only three specimens, ranging from 17 to 19 mm., were taken. The 1930 spat then ranged up to 8 mm. with an average size of 4·3 mm., and both its size-frequency and average size were similar to those of the 1929 spat.

Similar remarks apply to the other stations. At Stn. 8, for example (Table II), in September, 1929, there was a group of small individuals ranging from 3 mm. to 5 mm. By the end of May, 1930, the range was from 4 mm. to 13 mm. and the average size was 8·6 mm. A month later the range was from 7 mm. to 16 mm. and the average size 10·7 mm. No further collection was made until October, when only four specimens of the 1929 brood, 15 mm. to 19 mm., and only two specimens of the 1930 brood were taken. The collection taken in the beginning of December contained only seven specimens of the 1930 spat and none of the 1929 spat.

At Stn. 13 also a group of small individuals ranging from 3–7 mm. was present in September, 1929. By the end of May the range was from

5 mm. to 14 mm. and the average size was 8·1 mm. A month later the range was from 6 mm. to 15 mm. and the average size was 10·9 mm. In the beginning of October only one specimen of the 1929 spat was taken, but the new 1930 spat was present as a group of small individuals from 3 mm. to 5 mm. In the collection taken in the beginning of December the 1929 spat was found to range from 15 mm. to 19 mm. The range of the 1930 spat had increased and was from 3 mm. to 8 mm., but most of the specimens were under 7 mm. in length.

Stn. 18 was only sampled in the autumns of 1929 and 1930. Both in range and average size the spats of the two years were very similar, that of 1930 being, on the whole, rather more numerous.

TABLE II.

Stn. 8.

Date.	Length in mm. 3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	Aver. size 1929 spat.	Aver. size 1930 spat.
9.29	2	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3·8	.
26.5.30	.	1	4	10	18	21	20	23	10	4	2	.	.	.	.	.	.	8·6	.
29.6.30	.	.	.	.	2	1	2	7	5	6	2	.	.	1	.	.	.	10·7	.
4.10.30	1	.	1	.	.	.	.	.	.	.	.	.	1	1	.	1	1	.	.
12.12.30	6	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3·1

Stn. 13.

Date.	Length in mm. 3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	Aver. size 1929 spat.	Aver. size 1930 spat.
9.29	4	5	4	1	1	.	.	.	.	.	.	.	.	.	.	.	.	4·3	.
26.5.30	.	.	2	5	3	9	3	.	2	2	.	1	.	.	.	.	.	8·1	.
29.6.30	.	.	.	2	1	1	1	1	1	2	2	3	1	.	.	.	.	10·9	.
4.10.30	4	4	3	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	3·9
12.12.30	6	33	17	17	2	1	.	.	.	.	.	.	2	2	2	.	1	.	4·7

Stn. 18.

Date.	Length in mm. 3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	Aver. size 1929 spat.	Aver. size 1930 spat.
12.9.29	28	8	3	1	2	1	.	.	.	.	.	.	.	.	.	.	.	3·7	.
4.10.30	25	59	23	9	2	.	.	.	.	.	.	.	1	1	.	.	.	.	4·2

These results indicate that at Millport the cockle breeds in summer and that the spat is to be found on the ground towards the end of August. Of the spat some few individuals may reach a size of 10 mm. before winter, but most are under 6 mm. The winter ring laid down by these small shells is usually visible if looked for carefully. It is easily seen in young specimens up to one or two years of age, but in older shells, especially if there has been any erosion, the ring is often quite obliterated.

Further evidence confirming the time of spatting has been got from the state of the reproductive organs which, at Millport, seem to be ripe from about the end of July. Spawning has only once been observed. Some cockles taken on the 1st July, 1930, were put in a jar and next day, on

being given fresh water, proceeded to emit clouds of eggs and sperms. The sperms were active and clustered round each egg, but further development did not take place.

Reference has already been made to Orton's paper (**2**). Much of what he says is applicable to the Millport cockles, the great point of divergence is in the estimate of what constitutes the first winter ring. Orton in his figure (**2**, p. 251) undoubtedly calls the *first distinct ring* on the shell, apart from marking rings, the first winter ring, whereas in the Millport specimens this is the *second* winter ring. His experiment, however, while suitable for studying the growth of Cardium was not suitable for studying its spat fall. The experiment was conducted by placing marked cockles of varying size in a box moored in the river, and, to allow fresh water to flow through, the sides were covered with material having perforations 6 mm. in diameter. The box was opened periodically and the cockles examined. During spring and summer, in addition to the original large cockles, Orton found a number of small ones. These he assumed were spat carried in by the currents. While there is a possibility that this may have been the case, the more probable explanation is that these small cockles were some of the previous autumn brood which had either been washed into the box or had climbed in. The box was near the bottom of the river and it was quite possible for material from the bottom to be washed in as Orton admits (**2**, p. 253). The possibility that the small cockles may have climbed in is not at all improbable as the following observation shows. A number of small cockles about 4 mm. in length, were placed in a dish containing sea-water. One of these climbed up the side of the vessel to a height of about 2 cm. above the water, and repeated this twice when pushed back. The perforations in Orton's box were 6 mm. in diameter and, at Millport at least, even in early summer it is possible to find cockles of the previous autumn spat at this size. The smallness of the immigrating cockles, therefore, does not necessarily point to their being new spat. The question of time of spat fall is important, since on it depends the correct reading of the rings, and it would be of great value to have the question reinvestigated at Plymouth to decide if the breeding period of cockles in the two areas is really so different.

(*c*) DISTURBANCE RINGS.

Orton found that a ring was formed on the shell whenever the cockles were taken out and marked. These "false" or "disturbance rings" were similar in appearance to the true winter rings, and would have been read as such by anyone unacquainted with the history of the particular specimen. Practically all the cockles taken at Millport were of the same type, that is, they had the first two or three obvious rings on the shell separated by fairly large intervals. Only some half-dozen specimens

departed from this type in that instead of one ring there were two very close together. These are similar to the disturbance rings figured by Orton. In the cockle beds from which the collections were taken it would appear that disturbance rings are only found in a small percentage of the shells.

(*d*) AGE-COMPOSITION OF THE CARDIUM ON THE HUNTERSTON SANDS.

The material on which this and the subsequent paragraph are based consists of the quantitative collections from the regular stations, supplemented by three large collections from an unmeasured area of shore at Stns. 8 and 21. In addition, two other collections from the Hunterston Sands and one from Kilchattan Bay in Bute are included since they throw light on some particular point. The numbers are not sufficient to allow of any estimate of the rate of mortality or of the proportions of the various year-groups in the population before and after spatting. The collections from each station have been considered as a whole. Some few of the specimens in which the number of rings was not clear, e.g. those showing disturbance rings, have been left out of consideration.

TABLE III.

SHOWING THE PROPORTION OF EACH YEAR'S SPAT IN THE COLLECTIONS FROM EACH STATION CONSIDERED AS A WHOLE.

Stn.	1920.	1921.	1922.	1923.	1924.	1925.	1926.	1927.	1928.
8	.	.	.	.	7	17	189	19	24
13	.	.	2	1	6	15	26	10	17
18	.	1	.	1	1	10	18	5	4
21	1	4	8	8	14	21	279	57	40

Specimens spatted in all years from 1920 were taken and the numbers belonging to each year-group are set out in Table III, the spat of the years 1929 and 1930 excepted since they have been fully dealt with already.

The group spatted in 1926 was the dominant group at all stations, especially at Stns. 21 and 8 in the middle of the beds where density is greatest. At Stns. 13 and 18, at the landward and seaward edges of the beds, it was almost rivalled in numbers by the survivors of the 1925 spat. A supplementary collection from a position 100 yards landwards from Stn. 21 yielded 27 cockles from $\frac{1}{4}$ sq. m. of ground, and of these 21 were spatted in 1926.

This preponderance of the 1926 spat is significant when compared with the similar predominance of the 1926 spat of Tellina in Kames Bay. The observations on Tellina were made in a very small area, but the figures for Cardium are drawn from stations about a mile apart and on the other

side of the channel from Kames Bay. Taking the two sets of results into consideration the summer of 1926 seems to have been unusually favourable for the spatting of both Cardium and Tellina, and possibly other species as well. The causes of these variations have not yet been determined.

The predominance of the cockles spatted in 1926 over the other year-groups during the period 1926–1930, seems to have extended beyond the comparatively small area on the east side of the Cumbrae covered by these experiments. On the opposite side of the Cumbrae, and separated from it by about 3 miles of sea, lies Kilchattan Bay in Bute. A collection of cockles from near H.W.M. in the spring of 1929 gave the following distribution of year-groups :—

1924 spat . . .	11
1925 „ . . .	3
1926 „ . . .	15
1927 „ . . .	2
1928 „ . . .	0

This would point to the survival of the 1926 spat in unusual quantities over a fairly wide area. It is not possible at present to say just how far that area extended.

(*e*) SIZE AT EACH WINTER RING.

The size of each winter ring in the survivors of the successive broods has been calculated, but, except in the case of the 1926 spat, the numbers are so small that only a general summary has been given.

1930 *Spat.* (Tables I and II.)

In the beginning of August this was found at Stn. 21. The other stations were not examined at this time, but this spat would certainly have been found at them also. By the beginning of October at Stn. 21 the range extended up to 8 mm., but most of the specimens were not over 5 mm. in length. At Stn. 18 the range in October extended up to 7 mm., as at Stn. 21, however, most of the specimens were under 5 mm. At Stn. 13 only a few specimens ranging up to 5 mm. were taken. At Stn. 8 the October collection yielded only a few spat, and its scarceness at this station was emphasised by the December collection, when only 7 specimens were taken from $\frac{1}{4}$ sq. m. of ground. On the other hand, a collection taken at Stn. 13 in December gave 76 specimens per $\frac{1}{4}$ sq. m. These ranged up to 8 mm. in length, but the majority were below 6 mm.

1929 *Spat.* (Tables I and II.)

The individuals belonging to this group, like those spatted in 1930, showed no well-marked ring, only the faint first winter ring being visible. The first clear ring would be laid down in the winter 1930–31. Briefly

summarised -this group spatted in the late summer of 1929 was found at all stations in September of that year, ranging up to 8 mm. in length. During the following year it gradually decreased in abundance and was comparatively scarce by the end of 1930, the survivors then being between 15 and 19 mm. in length.

There is naturally a certain amount of variation from sample to sample, but during the year the numbers steadily declined and this was confirmed by an examination of the shore in October when the empty shells were lying everywhere. This raises the question as to whether the modes and means at various periods of the year, e.g. Tables I and II, represent the actual rate of growth of the survivors. I think they do not and that they are consistently too low. Some individuals of a group grow quickly from the start, others slowly, and it seems to be amongst the slower growing members that mortality is greatest.

1928 *Spat.* (Table IV.)

The cockles spatted in 1928 and taken during 1930 had only one well-defined ring on the shell, namely, the 1929–30 winter ring. They were not very plentiful. At Stn. 18 only four specimens were taken, and need not be considered. Those taken at Stns. 21 and 8 were alike, in that the range was large, 15–25 mm. and 14–26 mm. respectively, and the means much the same, 20·6 mm. and 20·3 mm., but differed from those taken at Stn. 13 where it was small, 15–20 mm., and the mean low, 17·4 mm.

TABLE IV.

Showing the Size-Frequency in mm. of the 1929–30 Winter Ring in the 1928 Spat.

Stn.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	Mean.
21	.	1	1	.	2	10	4	7	7	4	3	1	.	20·6
18	.	.	1	.	1	.	.	.	.	.	2	.	.	.
13	.	5	1	2	2	5	2	.	.	.	.	.	.	17·4
8	1	.	2	1	4	3	3	2	.	3	2	2	1	20·3

1927 *Spat.* (Table V.)

The individuals derived from this spat and taken in September, 1929, had only one well-marked ring, the 1928 29 winter ring, while those taken during 1930 had two, the 1928 -29 and 1929–30 winter rings. This was the scarcest of all the recent year-groups and, except at Stn. 21, only a few were taken.

1928–29 *Winter Ring.*

At Stn. 21 the range was from 14 mm. to 27 mm. and the mean was 20·8 mm. At Stn. 8 the range was rather narrower, but the mean was

about the same. At Stn. 13 the range was narrower and the mean less than at either of these two stations.

1929–30 *Ring*.

At Stn. 21 the range was from 22 mm. to 37 mm. and the mean was at 29·8 mm. At Stn. 13 the range was narrower, 24–28 mm., and the mean less, 26·4 mm.

TABLE V.

Showing the Size-Frequency of the 1928–29 and 1929–30 Winter Rings in the 1927 Spat.

1928–1929 Ring.

Stn.	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Mean.
8	.	.	.	1	1	.	2	6	3	1	1	.	.	.	.	.	20·9
13	.	.	1	.	1	2	1	4	1	.	.	.	.	.	.	.	19·6
18	.	.	.	.	.	.	.	2	1	.	.	1	1	.	.	.	.
21	2	.	1	4	4	9	7	6	9	7	4	1	2	1	.	.	20·8

1929–1930 Ring.

Stn.	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	Mean.
8	.	.	.	.	.	1	1	2	5	2	1	.	1	1	.	.	30·4
13	.	.	1	2	1	4	2	.	.	.	.	.	.	.	.	.	26·4
18	.	.	.	.	.	.	1	2	.	.	.	1	1	.	.	.	.
21	1	.	1	2	7	6	6	9	4	3	2	6	1	3	2	3	29·8

TABLE VI.

Showing the Size-Frequencies of the Winter Rings for the 1926 Spat.

1927–28 Ring.

Stn.	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean.
8	1	.	.	3	5	5	16	21	33	22	23	11	26	8	11	1	2	1	22·3
13	1	.	.	1	1	5	5	7	3	1	1	1	.	.	.	.	.	.	19·3
18	.	.	.	.	.	.	.	3	2	3	2	6	1	1	.	.	.	.	22·7
21	.	2	.	1	5	7	18	38	30	54	53	28	24	13	4	2	.	.	22·4

1928–29 Ring.

Stn.	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	Mean.
8	.	.	1	2	3	7	17	17	26	25	26	18	14	16	11	3	2	1	31·2
13	1	4	1	6	4	2	4	2	2	.	.	.	.	.	.	.	.	.	25·2
18	.	.	.	.	.	1	2	4	4	3	1	.	.	2	.	1	.	.	30·6
21	1	.	2	3	6	8	27	43	50	57	40	20	15	5	2	.	.	.	30·4

1929–30 Ring.

Stn.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	Mean.
8	.	.	.	.	.	1	8	11	14	21	31	26	23	20	9	17	3	3	.	2	36·0
13	1	3	2	5	4	2	2	4	2	1	.	.	.	.	.	.	.	.	.	.	29·3
18	.	.	.	.	.	1	1	4	5	1	1	2	.	2	.	1	.	.	.	.	33·9
21	.	.	.	1	.	7	21	31	40	46	52	41	27	5	5	2	1	.	.	.	34·2

1930–31 Ring.

Stn.	33	34	35	36	37	38	39	40	41	42	Mean.
8	3	3	3	3	5	3	3	1	.	1	36·5
18	1	1	.	3	1	2	.	.	.	.	36·0

1926 *Spat.* (Table VI.)

The specimens taken during September, 1929, had two well-marked rings, and those taken during 1930 had three well-marked rings. The length of specimens taken October and December, 1930, may be taken as the equivalent of the 1930–31 winter ring, which would just be beginning to be laid down. The size-frequencies and means of the various winter rings are set out in Table VI.

1927–28 *Winter Ring.*

At Stn. 8 the range was from 13 to 30 mm., but in most of the specimens was between 19 and 27 mm., and the mean size was 22·3 mm. At Stns. 13 and 18 comparatively few specimens were taken and on these the range was from 13 to 24 mm. with the mean at 19·3 mm. in the first case, and from 20 to 26 mm. with the mean at 22·7 mm. in the second case. At Stn. 21 the range was from 14 to 28 mm., but in most of the specimens was between 18 and 27 mm., and the mean was at 22·4 mm. The means at Stns. 8, 18, and 21 were all very nearly the same, but the mean at Stn. 13 was considerably lower.

1928–29 *Winter Ring.*

At Stn. 8 the range was from 24 to 39 mm., but as before most of the measurements were confined within narrower limits, namely, 28 to 36 mm. The mean was at 31·2 mm. At Stn. 13 the range was from 22 to 30 mm. and the mean was at 25·2 mm. At Stn. 18 the range was from 27 to 37 mm. and the mean was 30·6 mm. At Stn. 21 the range was again large, being from 22 to 36 mm.; as before the majority of the rings were confined within narrower limits, namely, 28 mm. to 34 mm. The mean was at 30·4 mm. As before the mean at Stn. 13 was considerably below the means of the other stations.

1929–30 *Winter Ring.*

At Stn. 8 the range was from 30 to 44 mm., but most of the figures lay between 32 and 40 mm., with the mean at 36·0 mm. At Stn. 13 the range was from 25 to 34 mm. and the mean was at 29·3 mm. At Stn. 18 the range was from 30 to 40 mm. and the mean was at 33·9 mm. At Stn. 21 the range was from 28 to 41 mm., but most of the figures lay between 30 and 38 mm., with the mean at 34·2 mm.

1930–31 *Winter Ring.*

The October and December measurements of the lengths of the shell may be taken as representing approximately the size of this ring. Since only very few specimens were taken at Stns. 13 and 21 these stations have not been included in Table VI.

At Stn. 8 the range was from 33 to 42 mm. and the mean was at 36·5 mm. At Stn. 18 the range was from 33 to 38 mm. with the mean at 36·0 mm.

Spat of 1925 *and Previous Years.* (Table VII.)

Very few of the cockles spatted in these years were found in the collections, and no useful purpose would be served by considering the size-frequencies of the various winter rings in detail, but for comparison the means of the various winter rings have been calculated. Table VII.

TABLE VII.

Table Showing the Mean Size of the successive Distinct Rings on each Year's Spat at the various Stations. Where only one or two Specimens were present the Average has not been calculated. At Stns. 8 and 13 the presumed Length of the 1930–31 Ring is included. The Material was Collected during 1929 and 1930.

				STN. 21.					
1921	20·5	30·2	35·8	39·2	42·0	44·5	46·0	47·2	.
1922	22·4	32·4	37·0	40·1	42·9	44·9	46·2	.	.
1923	22·2	32·1	37·2	40·3	42·8	44·1	.	.	.
1924	22·7	32·5	37·2	39·3	40·6	.	.	.	.
1925	23·0	33·0	35·6	39·2	.	.	.	.	.
1926	22·4	30·4	34·2	.	.	.	.	.	.
1927	20·8	29·8	.	.	.	.	.	.	.
1928	20·6	.	.	.	.	.	.	.	.
				STN. 18.					
1925	23·6	33·2	35·9	38·1	.	.	.	.	.
1926	22·7	30·6	33·9	.	.	.	.	.	.
				STN. 13.					
1924	22·8	28·7	31·7	33·8	35·7	38·0	.	.	.
1925	19·8	25·5	28·7	31·1	.	.	.	.	.
1926	19·3	25·2	29·3	.	.	.	.	.	.
1927	19·6	26·4	.	.	.	.	.	.	.
1928	17·4	.	.	.	.	.	.	.	.
				STN. 8.					
1924	25·9	35·6	39·3	41·3	42·6	.	.	.	.
1925	21·1	28·2	33·1	35·4	37·8	.	.	.	.
1926	22·3	31·2	36·0	36·5	.	.	.	.	.
1927	20·9	30·4	.	.	.	.	.	.	.
1928	20·3	.	.	.	.	.	.	.	.

In all but the 1926 year-group the numbers are too few to allow of much reliance being placed on the means, especially considering the wide range of the size-frequency figures for any one ring, e.g. Tables I, II, and IV, but the figures are fairly consistent. For any one station the means of the older groups are distinctly higher than those of the younger groups, and this would point to the suggestion already made that mortality tends to be heaviest at the lower end of the size-frequency scale.

It is interesting to compare this table with Orton 2, p. 250, Table II, showing the rate of growth of his marked cockles. Their rate of growth corresponds fairly well with that at Stn. 21, i.e. an average section from a natural bed and, except for the difference in the estimation of what constitutes the first winter ring, the two investigations are confirmatory.

In addition to the collections from the regular stations two others from these sands may be mentioned. One sample taken on the 16.8.30, from a position 100 yards landwards from Stn. 21, contained 27 cockles per $\frac{1}{4}$ sq. m. This position was roughly intermediate between Stns. 21 and 13 as regards level and, as was to be expected, both the range and means were intermediate between those of these two stations, e.g.

1927–28 Ring.	1928–29 Ring.	1929–30 Ring.
Range 17–24 mm.	Range 23–31 mm.	Range 27–33 mm.
Mean 20·5 mm.	Mean 27·8 mm.	Mean 30·5 mm.

Another collection was taken in July at some distance to the seaward of Stn. 21 beyond the cockle beds proper, and in the Tellina ground where only few and large cockles occur. (Stn. 16.) A $\frac{1}{4}$ sq. m. sample yielded 7 cockles which were remarkable for their large growth, e.g. Table VIII.

TABLE VIII.

1925–6 Ring.	1926–7 Ring.	1927–8 Ring.	1928–9 Ring.	1929–30 Ring.	Total length 1.7.30.	Spatted in autumn of	
33	44	52	53	54	54	1924	1928 Spat.
.	.	.	27	38	44	1927	1929–30 Ring.
.	.	.	24	35	39	,,	Mean 27·3 mm.
.	.	.	.	27	37	1928	
.	.	.	.	29	35	,,	
.	.	.	.	27	34	,,	
.	.	.	.	26	33	,,	

Kilchattan Bay, Bute.

This collection, already referred to on p. 284, was taken close to H.W.M., 29.3.29, and the means would be expected to compare with those of Stn. 13. This is actually the case, as may be seen by comparing with Stn. 13 the

specimens spatted in 1924 and 1926, of which 11 and 15 specimens, respectively, were taken.

	1925–26 Ring.	1926–27 Ring.	1927–28 Ring.	1928–29 Ring.
Stn. 13. Hunterston 1926 spat	.	.	19·3	25·2
Kilchattan Bay 1926 spat	.	.	17·9	25·7
,, ,, 1924 spat	20·0	25·8	28·8	31·5

(*f*) DIFFERENCES IN THE RATE OF GROWTH BETWEEN H.W.M. AND L.W.M.

It has already been shown (Stephen, **5**) that the cockle gets smaller from L.W.M. to H.W.M. This could come about in two ways, either by the rate of growth being different at various levels, or by the spat falling in greatest quantity on the upper part of the beach and gradually migrating seawards. The first explanation seems to be the correct one if the means are compared. If migration took place the means of the rings formed in the earlier years of life should be very much the same for all specimens and at all levels, actually the means increase from H.W.M. to L.W.M. Although the following four stations do not lie in a straight line their means may be used to illustrate this difference of growth. The means for the first three stations are those of the 1926 spat and for Stn. 16 that of the 1928 spat. Table IX.

TABLE IX.

	1st Clear Ring.	2nd Clear Ring.	3rd Clear Ring.
Stn. 13. Near H.W.M. . . .	19·3	25·2	29·3
100 yards up from Stn. 21. $\frac{3}{4}$ tide .	20·5	27·8	30·5
Stn. 21, half-tide	22·4	30·4	34·2
Stn. 16, near L.W.M. . . .	27·3	.	.

The cockle therefore increases regularly in size from H.W.M. to L.W.M. In this it resembles *Arenicola marina* and differs from *Tellina tenuis* and *Donax vittatus*.

So far as could be seen almost all the cockles at any station were of the same type, but occasionally at the lower stations a cockle was found which had obviously been washed down from higher up. At Stn. 21, for example, only three of the cockles taken were exceptional in that they had the annual rings much closer together than the rest of the specimens, and corresponded to the type found at the station 100 yards further up the beach.

This summary deals only with some of the more important points in the biology of the cockle, but others are being investigated, and it is hoped at a later date to deal with the species in greater detail.

3. Tellina tenuis.

Observations in Kames Bay have been carried out regularly during the period middle of September, 1926, to end of August, 1930, and while there is little to add to the accounts already published of the rate of growth and time of spat-fall (Stephen, **3** and **4**), new facts have been gathered regarding (*a*) the variations in the quantity of spat and in the total population from year to year, and (*b*) the growth and final disappearance of the generation spatted in the autumn of 1926.

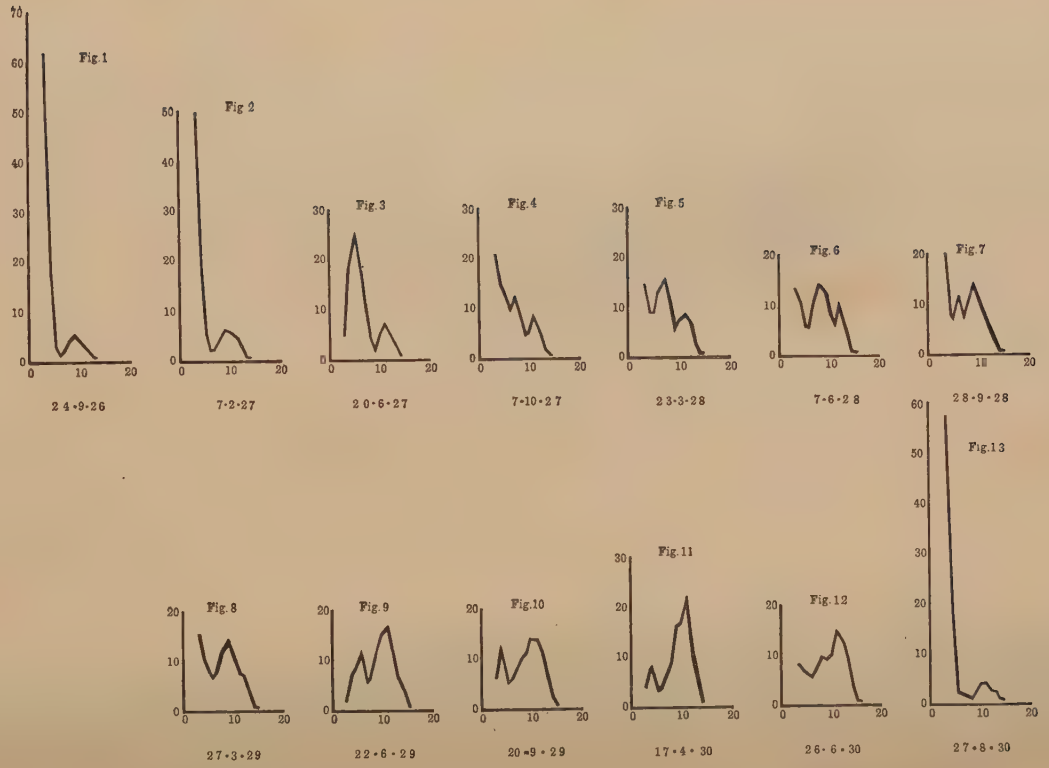
(*a*) Variations in the quantity of spat and in the total population.

In the earlier part of the investigation on *Tellina tenuis* in Kames Bay, collections were made at several stations, which were later reduced to two, namely, Stn. 2 near H.W.M. neaps and Stn. 5 at L.W.M. springs. Early in 1930 heavy storms completely altered and destroyed much of the upper part of the beach. The continuity of the experiment at Stn. 2 was therefore broken, but fortunately Stn. 5 was not affected, and a complete record of the size-frequencies of the population from September, 1926, to the end of August, 1930, has been obtained. Regular collections were made in spring, summer, and autumn, and in all but two cases these were quantitative. On the two occasions the station was not completely uncovered by the tide and qualitative collections only were obtained. A few supplementary collections were taken from time to time.

The amount of "spat" per unit area in Kames Bay in the autumn of each year is given in Table X A, all specimens 3 and 4 mm. in length being counted as "spat." These figures are probably a slight underestimate.

Spat was not laid down in equal amounts each year. In the autumn of 1926 it was very abundant and formed about 80% of the population. In 1927 it was also abundant, but the amount was only about half that of the previous year. Owing to the high rate of survival of the 1926 spat, however, it only formed 36% of the population. In 1928 spat was fairly plentiful, but again owing to the large number of older individuals on the ground formed only 28% of the population. Spat was very scarce in 1929, reaching the comparatively low figure of about 230 per sq. m.; 1930 was once more a year of abundance, although the number of spat was below that of 1926. The older individuals seem to have died out very largely after spatting, and the percentage of spat to total population once more rose nearly to the 1926 figure of 80%. These changes are shown in Figures 1 to 13.

There are two seasons when mortality might be expected to be high, (*a*) in winter amongst the spat and (*b*) in summer after spatting, amongst the older specimens. Winter mortality amongst the spat seems to be comparatively slight. In Table X B the percentage of spat to total population



FIGS. 1-13.—Showing the size-frequency of the *Tellina tenuis* population at Kames Bay, Millport, from 1926-30. The Ordinates represent the percentage of the catch and the abscissæ the sizes in mm.

has been tabulated for autumn and spring. In each case the spring percentage is only a little less than the autumn one, and it is evident that the death-rate has been low. In the second case mortality after spatting is often very heavy, and a whole year-group may die off within a short period (pp. 294, 297).

TABLE X.

Showing the Percentage of Spat to Total Population in Autumn and Spring at Stn. 5, Kames Bay, Millport.

Date.	No. of Spat per sq. m.	Total Tellina Population per sq. m.	% of Spat.
A. AUTUMN.			
24.9.26	2620	3288	80
7.10.27	1088	3056	36
28.9.28	854	3038	28
20.9.29	232	1348	17
27.8.30	1628	2136	76
B. SPRING.			
13.4.27	2664	3595	74
23.3.28	716	2912	25
27.3.29	508	2004	25
17.4.30	156	1480	11

(*b*) VARIATION IN DENSITY OF POPULATION.

In accordance with the variation in recruitment from unequal spattings coupled with the rise and decline of the 1926 spat, the density of population at Stn. 5 has fluctuated considerably during the period, as may be seen from Table XI.

TABLE XI

Showing the Variation in Density of Population per sq. m. at Stn. 5. (Owing to the great similarity of the figures for each of the collections during 1927 and 1928 the table has been shortened by giving the average for these years.)

24.9.26	3288 per sq. m.
1927 (average)	3403 ,, ,,
1928 ,,	2883 ,, ,,
27.3.29	2004 ,, ,,
20.9.29	1348 ,, ,,
17.4.30	1480 ,, ,,
26.6.30	1094 ,, ,,
27.8.30	2136 ,, ,,

In the autumn of 1926 the density of population was over 3000 per sq. m. During 1927 the figure was very much the same and dropped only slightly during 1928. From the autumn of 1928 onwards the fall was rapid, till by the middle of 1930 the density was just over 1000 per sq. m. In August the appearance of the new 1930 spat in large quantities almost doubled the density.

(*c*) RISE AND DECLINE OF THE 1926 SPAT.

As already stated, spat appeared in abundance in the autumn of 1926 and the survivors continued to be the dominant year-group until the summer of 1930, when, after spatting, they largely died out. This has been illustrated graphically in Figs.. 1–13. The figures from which these curves are drawn are given in Stephen, **3**, for 1926–27, Stephen, **4**, for 1928, and in Table XIII for 1929–30.

Judging by the modes, Table XII, this group seems to have reached its maximum size by the summer of 1929.

TABLE XII.

SHOWING THE MODES OF THE 1926 SPAT AT STN. 5 FOR EACH TIME OF COLLECTION, 1926–1930.

24.9.26	3 mm.	27.3.29	9 mm.
13.4.27	3 mm.	22.6.29	11 mm.
20.6.27	5 mm.	20.9.29	11 mm.
7.10.27	7 mm.	17.4.30	11 mm.
23.3.28	7 mm.	26.6.30	11 mm.
7.6.28	8 mm.	27.8.30	11 mm.
28.9.28	9 mm.		

The story of the 1926 spat may therefore be summarised as follows :— Spatted in the late summer of 1926, it was found in quantity at Stn. 5 in September, forming a well-defined peak on the size-frequency curve and comprising about 80% of the population. The modal size in September, 1926, was 3 mm., and by the autumn of 1927 this had increased to 7 mm. By the autumn of 1928 it had reached 9 mm., and by June, 1929, 11 mm., at which figure it remained until the following year. In the first half of 1930 the ground was very largely populated by the 1926 spat, but by August the character of the population was completely changed. The individuals spatted in 1926 had largely died out and the 1930 spat had appeared in quantity. The percentage of spat to total population was 76%, that is, approximately, the 1926 figure, after an interval of four years.

The fate of the spat of the intervening years 1927–29 is of interest since the curves (Figs. 1–13) are largely bimodal and give the false impression that there are only two year-groups on the ground.

In the autumn of 1927 spat came down in fair abundance and showed on the curves for the whole of the next year. The mode was at 3 mm. in October, 1927 (Fig. 4), and March, 1928 (Fig. 5) ; 3 mm. in June (Fig. 6) ; and 6 mm. in September (Fig. 7). From then onwards it seems to have disappeared entirely from the curves, having apparently failed to survive the winter.

In 1928 spat was also found in fair abundance. In September, 1928, it showed as a group with the mode at 3 mm. (Fig. 7). The mode was at the same figure in March, 1929 (Fig. 8). In June the mode was at 6 mm. (Fig. 9), and by September this spat had apparently overtaken the slowest growing individuals of the 1926 spat and the two curves merge into one, forming a composite group, the mode showing as an irregularity on the curve about 8–9 mm. (Fig. 10). This irregularity continued to show on the curves until June, 1930 (Figs. 11 and 12), and it is presumed that the 1928 spat continued to survive until then. The spat in 1929 was very scarce and could not be traced on the curves for 1930.

Rich spat-falls apparently occur only at intervals, and may produce a year-group which remains dominant on the ground for some years. Two factors probably combine to produce this result. There must, of course, be a specially abundant spat-fall, but the spat must also get a good start, for a poor start is apparently a handicap which is never overcome. For example, the spat of 1927 was more abundant than that of 1928, yet the spat of 1927 soon disappeared from the curves while the 1928 spat could be traced until June, 1930. A possible reason for this difference appears if the early history of each year-group is compared. Both ceased to grow during the winter and both started off with a mode of 3 mm. in spring, but whereas the mode of the 1927 spat was still at 3 mm. in June (1928) the mode of the 1928 spat was at 6 mm. in June (1929). By the end of 1928 the mode of the 1927 spat had only reached 6 mm. (equivalent to the June mode of the 1928 spat), while by the end of 1929 the 1928 spat had reached 8–9 mm. Apparently the adverse spring of 1928 had been a handicap to the 1927 spat from which it had never recovered. These interesting problems will be the subject of further investigation.

TABLE XIII.

Showing Percentage at each mm. size for Tellina tenuis at Stn. 5, Kames Bay, 1929–1930.

Date.	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total No. of specimens.
27.3.29	15·3	10·1	8·0	6·3	7·5	11·5	13·4	10·1	7·1	6·8	3·3	0·5	0·2	.	0·1	1002
22.6.29	1·8	6·7	8·0	10·6	5·0	6·2	10·6	14·1	15·9	11·8	5·9	3·8	0·3	.	.	341
20.9.29	5·8	11·3	4·6	5·3	7·6	9·6	10·3	13·5	13·4	11·1	5·5	1·7	0·4	.	.	1012
17.4.30	3·5	7·3	3·0	3·2	6·0	8·4	15·9	16·1	20·8	10·5	5·1	0·8	.	.	.	370
26.6.30	8·0	6·7	6·0	5·1	6·7	9·0	8·8	9·9	14·0	12·6	8·9	3·1	0·6	0·2	.	547
27.8.30	57·7	18·4	2·2	2·0	1·8	0·9	2·9	4·0	4·1	2·4	2·3	0·9	0·3	.	.	1068

4. Macoma baltica.

Rate of growth and time of spat-fall (figs. 14 and 15).

An attempt has been made to estimate the rate of growth and time of spat-fall for this species. It is not very abundant in the Cumbrae area, but is plentiful in certain parts of the Firth of Forth. In Aberlady Bay, for example, where the species is relatively abundant, the density reaches about 150 to 200 per sq. m. in places. This bay was therefore chosen as a

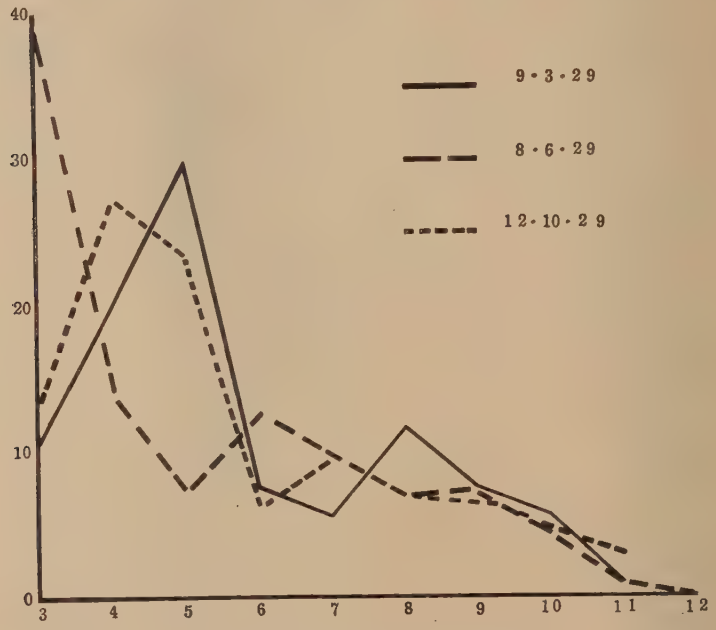


Fig. 14.—Showing the size-frequency curves for the collections of *Macoma baltica* taken in Aberlady Bay during 1929.

Ordinate percentage of the total catch.
Abscissa size in mm.

suitable locality for study, and collections were made during 1929 and 1930 at one station about 100 yards from H.W.M.

In the spring of 1929 there were modes at 5 mm. and 8 mm. on the curve, representing, it may be assumed in the light of later knowledge, the 1928 and 1927 spat respectively. In the beginning of June the modes were at 3 mm. and 6 mm. with a hint of a third at 9 mm. The mode at 3 mm. represents the new 1929 spat, that at 6 mm. the 1928 spat, and the mode at 9 mm. the 1927 spat. The latter seemed to be dying off after the

breeding season. In October the modes were at 4 mm. and 7 mm., the 927 group having apparently entirely disappeared.

Compared with the spring modes of 1929, which may be taken as the equivalent of the October, 1928, modes, since growth ceases during the winter, the position of the modes in October, 1929, would point to growth having been slower than in the previous year, probably on account of the heavier spat-fall and therefore greater competition for food.

In 1930 growth among the smaller individuals had begun before the

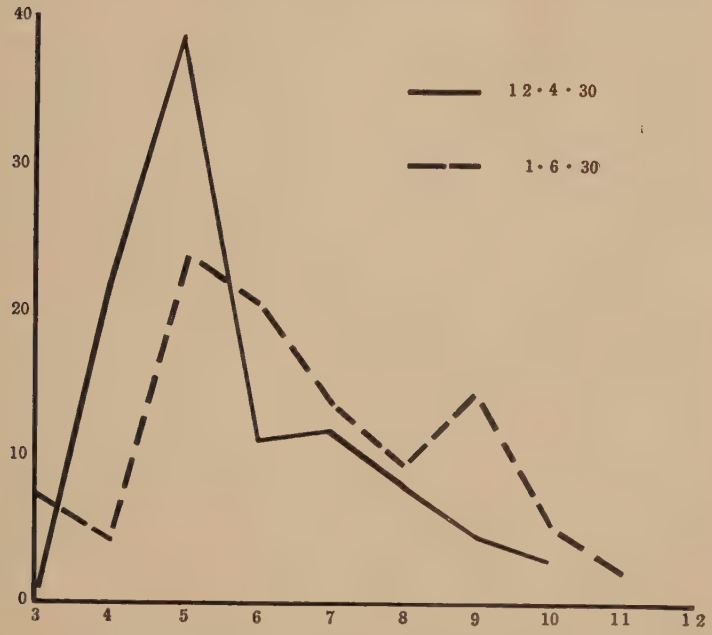


Fig. 15.—Showing the size-frequency curves for the collections of *Macoma baltica* taken in Aberlady Bay in 1930.

Ordinate percentage of the total catch.
Abscissa size in mm.

spring samples were taken, since the first mode had moved from 4 mm. to 5 mm. The second mode remained unaltered. On the June curve, as in the previous year, there were three modes at 3 mm., 5 mm., and 9 mm., representing the new 1930 spat, the 1929 spat, and the 1928 spat respectively. Sampling was repeated on two occasions in October, but for several reasons the collections were unsatisfactory. The size-frequency curve for these small collections gave a hint of modes at 5 mm. and 7 mm., but it was impossible to judge if the oldest group had survived.

Judging by the analogy of the previous year it had probably died out more or less completely after the breeding season.

To summarise. *Macoma baltica* apparently breeds in spring, and since spat was found in June of both the years 1929, 1930, the period April to May is suggested as the possible breeding period. The spat is well-grown before winter sets in. Judging by the modes the size at the end of the first autumn is about 5 mm. and at the end of the second about 7–8 mm. at this particular station. It would seem from the 1929 figures that mortality is heavy after spatting in the third summer. Breeding probably occurs earlier in life, but it is after this time that the large individuals tend to disappear in greatest numbers.

The year 1929 seems to have been a very favourable one for spat, since the average density of spat in June was about 150 per sq. m. On the other hand, the year 1930 seems to have been very poor, since the density in June of that year was only about 30 per sq. m. The term " spat " in the above paragraph has been restricted to individuals up to 3 mm. in length. The size-frequencies of the specimens taken in 1929 and 1930 are set out in Table XIV.

TABLE XIV.

Showing the Frequency at each mm. size for *M. baltica* in Aberlady Bay, Firth of Forth, during 1929 and 1930.

Date.	3	4	5	6	7	8	9	10	11	12	Total No. of specimens.
9.3.29	11·0	20·3	30·0	7·4	5·5	11·5	7·4	5·5	0·9	.	216
8.6.29	37·9	14·0	7·2	12·4	9·7	7·0	7·2	4·3	0·8	0·2	518
12.10.29	13·6	27·2	23·2	6·3	9·4	7·4	6·3	4·2	2·6	.	191
12.4.30	0·6	21·5	38·4	11·0	12·8	8·1	4·7	2·9	.	.	172
1.6.30	7·2	4·1	23·7	20·6	13·4	9·2	14·4	5·2	2·1	.	97

5. SUMMARY.

(*a*) CARDIUM EDULE OR COCKLE.

1. The observations were made on the Hunterston Sands, Ayrshire, opposite the Cumbrae.

2. Small cockles appear on the ground about the beginning of August. This points to the breeding season being in *summer* and not spring, as is usually stated.

3. By the end of the first autumn some few of the spat are as much as 10 mm. in length, but most are under 6 mm. The winter ring is faint and easily overlooked.

4. The first of the several well-defined rings is therefore not the *first* but the *second* winter ring.

5. Certain year-groups predominate. The specimens taken during 1929 and 1930 were largely the survivors of the 1926 spat.

6. The mean length of each of the winter rings has been tabulated for each year-group. These means tend to be higher in the older groups, pointing to mortality being heaviest amongst the slower growing individuals.

7. The size increases regularly from H.W.M. to L.W.M., due to an increased rate of growth. Growth is very fast amongst the scattered individuals living in the Tellina ground, that is, seawards of the cockle beds proper.

TELLINA TENUIS.

1. The variations in the annual spat-fall at Stn. 5 in Kames Bay from 1926–30 are traced. Some years give a rich spat, others only a poor spat, but the final contribution to the population may depend on other factors as well, e.g. a good growing start.

2. Mortality is comparatively light amongst the spat during winter, but may be exceedingly heavy after spatting amongst the adult population.

3. At Stn. 5 the group spatted in the autumn of 1926 remained the dominant year-group until the summer of 1930.

4. The spat of 1930 was abundant and, owing to the heavy mortality amongst the adults after spatting, the type of size-frequency curve seen in September, 1926, was repeated, after an interval of four years.

MACOMA BALTICA.

1. The species was observed in Aberlady Bay, Firth of Forth.

2. Small individuals appear in June and the breeding period is probably April to May.

3. By the end of the first year the average size is about 5 mm., and by the end of the second 8 to 9 mm. The end of the breeding season in the third summer seems to mark the termination of the life of the average individual.

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On the Effect of the severe Winter of 1928–1929 on the Oyster Drills (with a record of five years' observations on sea-temperature on the oyster-beds) of the Blackwater Estuary.

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With 2 Figures in the Text.

During the year 1928 the writer was engaged in a study of the bionomics of the oyster drills on the oyster beds in the region of the River Blackwater, Essex. At this time the presence of a foreign species was detected (1) and definite records were made of the relative abundance of the three following drills or tingles: -*Murex erinaceus* (=Ocinebra), *Purpura lapillus* (=Nucella), and *Urosalpinx cinerea*. It so happened that the winter of 1928–29 was unusually cold; the temperature of the water over the oyster beds was unusually low in January and February, 1929, ranging about the freezing-point of fresh water (see Table II and Fig. 1). The oyster-cultivators in this locality had many years previously stated that during severe winters many marine animals, such as the oyster drills or tingles and the burr *Echinus miliaris*, are killed in such great numbers as sometimes to exterminate the species. The observations made by the writer in 1928 offered an opportunity to test these statements, and of estimating the effect of the extreme cold in early 1929 on the animals mentioned above.

Three of the four species studied, namely, Murex, Purpura, and Echinus, are rarely found—in the district of the River Blackwater—above low-water mark; Urosalpinx may be taken by hand in fair numbers especially in the warmer months of the year on or about oysters at extreme low-water springs. (Such a habitat is noteworthy in the case of *Purpura lapillus*, which is abundant on the foreshore in the *Balanus balanoides* zone on the coasts of Devon and Cornwall, and in some other localities.)

The constant immersion during the winter period of the four species noted eliminates any direct effect of air temperature, and confines the influence of temperature to that of the water. Temperature readings at and about high and low water have been recorded almost daily for the writer by Mr. Laban Pearce (see **2**) over a period of five years, and are recorded in Tables VII, p. 313, I and II, and Fig. 1, p. 304, being reduced to mean monthly figures. These readings prove that the mean monthly

TABLE I.

MEAN MONTHLY TEMPERATURE OF SEA AND AIR OF THE BLACKWATER ESTUARY (IN DEGREES FAHRENHEIT).

	Air* (1876–1910) Blackwater.	Sea‡ (1926–8, 1930) Blackwater.				Air† (1928–9) Clacton-on-Sea.	Sea (1928–9) Blackwater.
		H.W.	L.W.	Mean °F.	Mean °C.		
Jan.	37·9	38·9	38·7	38·8	3·7	34·9	34·2
Feb.	39·3	40·6	40·3	40·4	4·6	31·9	32·5
March	41·7	43·6	43·9	43·7	6·5	41·1	38·9
April	47·0	48·5	48·8	48·8	9·2	43·5	45·1
May	52·4	54·9	55·5	55·2	12·9		
June	58·4	61·5	62·0	61·7	16·5		
July	62·1	65·8	65·8	65·8	18·7		
Aug.	62·4	64·3	64·2	64·3	17·9		
Sept.	58·0	61·8	61·3	61·6	16·4		
Oct.	51·1	53·2	52·5	52·8	12·6		
Nov.	44·4	46·0	45·3	45·6	7·5	1928	
Dec.	40·0	39·9	39·0	39·5	4·1	39·7	39·4

temperature of the water over the oyster beds in the River Blackwater was 4° to 10° F. below normal during most of January and February and the first week in March in 1929. The two-day means of readings (usually four) during January and February are also plotted in Fig. 1, p. 304, from the daily readings given in Table II.

The effect of this extreme cold is well shown in the analyses of catches of oyster drills made in 1928, 1929, and 1930, as given in Table III. Of the 10,852 living drills examined during the three years the percentages there shown were obtained. But, owing to the fact that the drills have different habits, it is necessary to discuss the relative abundance of the

* Mean of the monthly means of maximal and minimal readings at Clacton-on-Sea and Southend combined (see **8** M.O. 214a, App. IV).

† Means are only available for Clacton-on-Sea: temperatures at the more insular situations on the Blackwater are slightly lower in cold and higher in warm periods (see **7**, p. 213).

‡ Means of daily readings at about high and low water except Sundays. About 50 readings per month were made in most months. For a discussion on the significance of the readings see **2**, p. 387, and legend to Fig. 1, p. 305, herein.

animals on different types of grounds, in order to assess the significance of the figures obtained.

The environs of the mouth of the Blackwater River and the site of the oyster beds are shown in Fig. 2, p. 306. It was found in 1929 and 1930

TABLE II.

Daily Sea-temperature readings at Thornfleet* on the Blackwater Estuary during the cold period December 1928–March 1929 (to nearest degree Fahrenheit).

	L.W.	H.W.		L.W.	H.W.		L.W.	H.W.
Dec. 17	37	39	Jan. 18	32	33	Feb. 19	29	30
„ 18	37	38	„ 19	33	32	„ 20	29	30
„ 19	36	37	„ 21	32	33	„ 21	31	32
„ 20	37	36	„ 22	33	33	„ 22	31	32
„ 21	37	37	„ 23	34	34	„ 23	—	33
„ 22	38	37	„ 24	34	34	„ 25	31	32
„ 24	37	38	„ 25	34	34	„ 26	31	32
„ 25	—	39	„ 26	34	34	„ 27	30	31
„ 27	39	40	„ 28	32	34	„ 28	30	32
„ 28	40	40	„ 29	32	34	Mar. 1	30	32
„ 29	39	—	„ 30	34	34	„ 2	31	—
„ 31	37	37	„ 31	36	35	„ 4	33	32
Jan. 1	37	37	Feb. 1	37	35	„ 5	33	32
„ 2	36	37	„ 2	36	38	„ 6	35	34
„ 3	36	36	„ 4	35	36	„ 7	37	36
„ 4	35	35	„ 5	35	35	„ 8	38	36
„ 5	35	35	„ 6	36	35	„ 9	37	36
„ 7	33	34	„ 7	36	36	„ 11	37	36
„ 8	34	34	„ 8	37	36	„ 12	39	37
„ 9	36	35	„ 9	36	37	„ 13	40	37
„ 10	35	35	„ 11	34	33	„ 14	39	37
„ 11	35	35	„ 12	29	32	„ 15	38	37
„ 12	33	34	„ 13	29	31	„ 16	38	—
„ 14	38	35	„ 14	29	31	„ 18	38	38
„ 15	34	35	„ 15	28	30	„ 20	41	39
„ 16	33	35	„ 16	ice		„ 23	42	41
„ 17	33	34	„ 18	29	29			

TABLE III.

	Urosalpinx.	Murex.	Purpura.	No. Total.
1928	7·8%	41·2%	51·0%	1,739
1929	83·4%	0·1%	16·6%	1,184
1930	84·5%	0·1%	15·4%	7,927

that the grounds A_1, A_2, A_3, and A, which are headwaters or creeks, yielded only Urosalpinx. As these grounds had not previously been carefully surveyed, it is probable that Murex, and possible that Purpura, did

* Occasional readings, which were of the same order as those obtained at Thornfleet, were also made at the following stations:—Off Tollesbury Pier, West Boundary, South Shore. Readings made from Calderara thermometer with N.P.L. certificate and read to nearest degree.

not formerly occur there, except as occasional introductions. On the other hand, Urosalpinx had—for at least a few years before 1928 –been taken there in abundance. The captures for these "A" grounds are

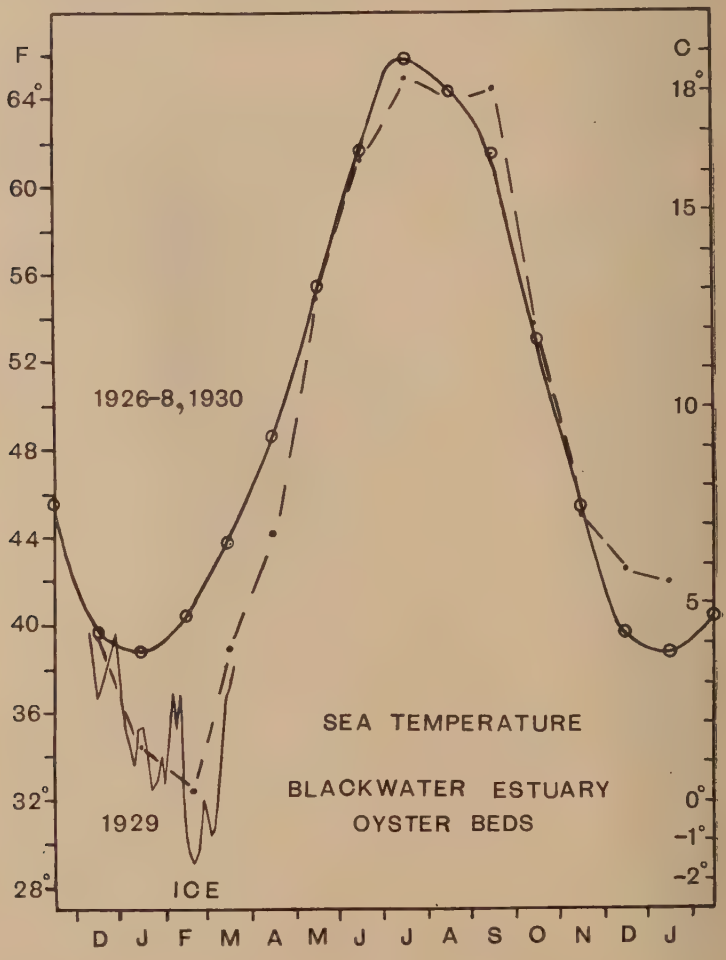

FIG. 1. (See foot of page 305.)

therefore listed separately in Table VI, p. 312, in order to permit of a direct comparison of the captures during the three years on those beds where both Murex and Purpura were known to occur in abundance in 1928. Thus, omitting the "A" grounds or creeks, the following percentages of the living tingles are obtained :—

TABLE IV.

	Urosalpinx.	Murex.	Purpura.	No. Total.
1928	7·8%	41·2%	51·0%	1,739
1929	71·2%	0·2%*	28·6%	584
1930	77·9%	0·11%*	22·0%	5,467

In this Table the yields of tingle in the three years are directly comparable and were all obtained by dredging. There can be no doubt, therefore, that after the winter of 1928–29 (*a*) Murex was almost eliminated from the beds, and (*b*) that Purpura most probably also became far less abundant, (*c*) that apparently Urosalpinx increased in abundance. The absence of any increase in the proportion of Murex and Purpura in 1930 virtually proves that these tingle were not driven into deeper water in the winter of 1928–29. There can be no doubt, therefore, that Murex did in fact suffer almost complete annihilation, and that serious mortality occurred among the Purpura. Since quantitative hauls were not made it is impossible to state that Urosalpinx actually increased in numbers, although it is probable that an increase occurred. The fishermen report that tingle as a whole were less abundant in 1930 than formerly.

Fig. 1.—Mean monthly temperature of the sea over the oyster beds in the Blackwater Estuary compiled from daily readings at a depth of about one metre (surface) at about high and low water (Sundays excepted) for the five years 1926–1930.

The means of the four years, 1926–28, 1930, are averaged to give the continuous thick-lined curve. The means for the year 1929 are shown by the discontinuous thick-lined graph; the means of readings (usually four) for each two days in the cold period, December to the middle of March, are shown in the continuous thin-lined graph.

The readings were taken at the temperature stations shown in circles Fig. 2, p. 306, and mainly at the Thornfleet Station. The number of readings at each station is as follows:—

		Depth in fathoms at low water.	No. of readings.
A_1	Thornfleet	2	2185
C_1	Tollesbury Pier=Mell Pier	3	325
E.B.	East Boundary	3	223
W.B.	West Boundary	3	197
M.	Marfleet	5	15
E.	South Shore	2	24
S.H.	Shingle Head	3	3
C_3	Nass End	2	27
B.	Deeps	2	67
A_4	*Dan's* Moorings	1	192
	Other stations.	–	6

Readings below the surface rarely differ from bottom temperatures (as determined by a Nansen-Pettersen water-bottle) by more than a fraction of a degree F., at the shallower stations, i.e. where the depth of water is about 2–3 fathoms at low water. Slightly greater differences may occur between surface and bottom when the depth is greater, i.e. 5 to 10 fathoms (see **6**, *loc. cit.*, p. 6).

* All individuals small to medium in size. See Table VI, p. 312.

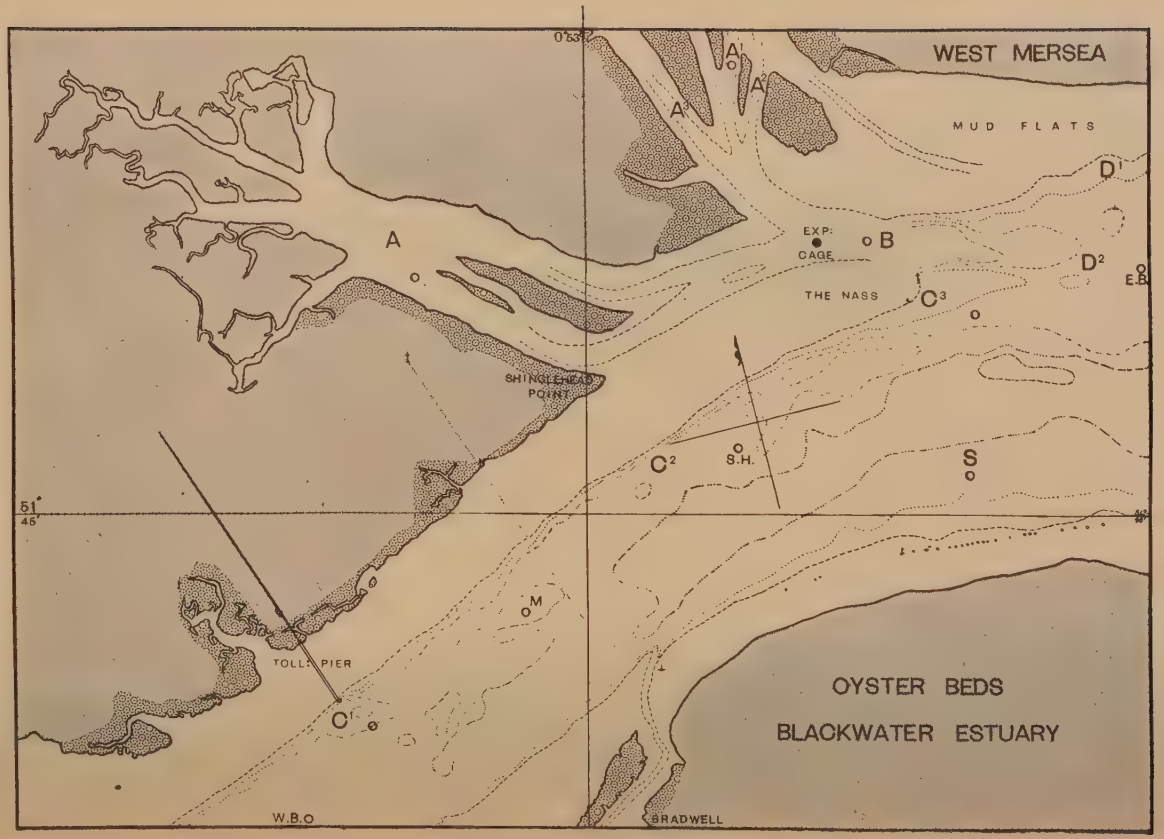

FIG. 2 (see foot of page 307).

Contributory evidence to these conclusions is offered by the figures of the dead tingle obtained with the living ones. In 1929 and 1930 the dead individuals were carefully separated from the living and counted. (To prove death it was necessary to break open the doubtful shells. It was then observed that Purpura can retract itself much further into the shell than Urosalpinx, and that very badly worn and apparently empty shells of Purpura may contain an exceedingly emaciated living animal.) The proportions of dead and living shells in the catches made in 1929 and 1930 are shown in Table VI, p. 312, and summarised below in Table V. The dead shells caught in 1928 were not recorded.

TABLE V.

Grounds.	Urosalpinx.		Murex.		Purpura.		Total dead.		Total
B, C, D.	No.	%	No.	%	No.	%	No.	%	living.
1929	16	3·7	25	96·2	17	9·2	58	9·0	584
1930	41	0·9	126	95·5	110	8·4	277	4·8	5,467

The " A " grounds may be excluded from consideration, as it is probable that they do not constitute a natural habitat of Murex and Purpura. On the remaining grounds the total number of each species taken dead in 1929 and 1930 is shown above, along with the percentage of dead shells calculated on the total number of living and dead shells of each species. The total number of dead shells of *all* species is also shown, as well as the total percentage dead of the total living and dead. The very high percentages of 96·2 and 95·5 Murex dead, and the greater actual number of Murex dead in comparison with the total dead of Urosalpinx and Purpura, both offer additional evidence of the virtual annihilation of the rough English tingle on the Blackwater oyster beds in the winter of 1928–29.

The great increase in the percentage of living Urosalpinx on grounds C, D, and E in 1929 and 1930, as shown in Tables II and III, is undoubtedly an indication of the greater capacity of this species to resist extreme cold.

FIG. 2.—Chart of the West Mersea oyster beds and temperature stations in the Blackwater Estuary. (From Admiralty Chart 3740, 1918.)

A_1	Thornfleet beds.
A_2	Mersea Fleet beds.
A_3	Salcott Fleet beds.
A	Tollesbury Fleet and M.V. *Dan's* Moorings.
B	Deeps.
Exp. cage	Site of experimental oyster cage.
C_1	Grounds off Tollesbury Pier (=Toll. Pier).
C_2	Grounds below Marfleet (M.) and off Shingle Head.
C_3	Grounds off the Nass End.
D_1	North shore ground near East Boundary (E.B.).
D_2	Offshore northern grounds (Flat Ground).
S.	South shore beds near watch boat.

Thirslet Creek lies a little above Tollesbury Pier and is not shown. The temperature stations (shown in circles) are denoted in the legend to Fig. 1, p. 305.

The virtual absence of Murex in the catches is at the same time an indication of the susceptibility of this species to low temperatures or conditions associated with low temperatures of the kind and duration shown in Table I; on the figures shown it is highly probable that Purpura also succumbed in considerable numbers under these unusual conditions.

Reviewing the oyster fishery as a whole it is clear that the American oyster tingle has now spread to all the grounds and has replaced the English tingles to a great extent; inasmuch as among nearly 8,000 individuals of all kinds captured in 1930, Urosalpinx occurred in percentages varying from 43 to 100 on the different beds and constituted 84% of the total captures. As Urosalpinx is known to be a voracious feeder on young oysters (**1**), the economic difficulties of prosecuting oyster culture on the Blackwater beds—already made difficult by the multiplication of the American slipper-limpet, *Crepidula fornicata* (**3**)—have been seriously increased. There is no evidence of unusual mortality among Crepidula on these beds as a result of the cold conditions noted, but as this species is exceedingly abundant, it is probable that a slight increase in mortality would pass unnoticed. Individuals were, however, observed to be weak and easily detachable from their chains.

The differential resistance of the specific protoplasms of (certain organs of ?) these species of Urosalpinx, Purpura, and Murex in the natural habitat to the low temperatures observed and recorded herein is apparently the cause of the selective mortality observed. It would seem that such a fundamental property of specific protoplasms is worthy of further experimental investigation on the lines of Huntsman and Sparks (**4**) and Battle (**5**).

The result of the study has proved that the observations of local naturalist-fishermen are correct as regards *Murex erinaceus* and (as will be shown later) *Echinus miliaris*, but incorrect concerning *Purpura lapillus* and *Urosalpinx cinerea;* but as the latter is not an English form and the actual date of its introduction into English waters is doubtful, it is certain that the American tingle has not been included in the consciousness of the local fishermen in the last 20 to 30 years. It is probable that *Murex erinaceus* was the dominant tingle on the Blackwater oyster beds until 20 to 30 years ago.

Echinus miliaris.

Echinus miliaris was abundant about and in the creek (Thirslet Creek) immediately above Ground C_1 (Fig. 2, p. 306) in 1913, but rarely occurred on any of the Blackwater beds in the post-war years. It is probable that this species was exterminated from these habitats in the cold winter of 1916–17, though it survived in smaller numbers on some deeper offshore beds. During the years 1920–28 it is known that this species occurred

in abundance on the oyster beds off Whitstable (**6**), and samples up to 500 or more have been utilised at intervals at Plymouth for transplantation, experimental work, and artificial fertilisations. In the spring of 1929, however, it was found impossible to obtain samples; it was reported that all the *Echinus miliaris* had been killed off during the winter on the shallower inshore grounds, but could still be obtained in small numbers in the deeper offshore waters, which are rarely worked.

The variation in the sea-temperature over the Whitstable oyster beds is comparable with that on the Blackwater beds, except that temperatures range somewhat higher in the summer and lower in the winter on the latter (**8**). There can be no doubt, therefore, that *Echinus miliaris* was exterminated as a result of the low temperatures of the winter of 1928–29 on the Whitstable oyster beds in the same way as Murex on the Blackwater beds.

It is impossible to state in what precise way the unusual reduction in temperature causes death in such instances as are noted above without experimental observations. The probable factors are:—the change in absolute temperature, the rate and period of fall or rise of temperature, and the correlated degree of salinity at different phases of change. No observations on salinity were made during the cold period, but information can be culled from data on the concomitant precipitations as compared with the normal. The monthly weather reports for the period, December 1928 to April 1929, show that the variations from monthly normals (**8**) in total rainfall (in mm.) were respectively December +39, January +6, February —19, March —32, and April +9. There was therefore an exceptional rainfall only in December (17 mm. on the 29th), some six to seven weeks before the water was reduced to freezing-point, and exceptional dryness in March. In the coldest period there was very little dilution of the sea-water by precipitation, and no heavy rains followed in March and April during the period of rapid rise in temperature. It would seem, therefore, that the mortality observed was due mainly either to the fall in absolute temperature or the rate of change of temperature at a low level, but that the reduction in salinity at the onset of the cold period may have predisposed the animals in some degree to a lethal effect of low temperature.

On Natural Selection.

It is interesting to note that the foregoing events comprise a minor cataclasm of the kind envisaged by Bidder (**9**). Among the three species studied, Urosalpinx and—to a less extent—Purpura survived the (locally) unusual environmental phases. Within the species of *Murex erinaceus* a high degree of mortality occurred, but a small proportion of individuals survived, and may be stated to have been naturally selected.

The surviving individuals which were captured, it is of great interest to note, were all either of small or medium size, that is, lengths in mm., 15.6.29, ca. 25 ; 19.5.30, 25·3 ; 23.4.30, 29·0, 20·8 ; 24.5.30, 32·2, 29·0, 25·3 ; giving an average length of about 27·0 mm. In 1928 a common size among the larger individuals was 40 to 45 mm. (See **11.**) It is permissible to speculate whether the surviving individuals avoided death by some peculiarity of their own or of their parents, and if so, whether that peculiarity might be heritable. There is, however, no critical evidence available on the subject in this instance, which may be regarded as typical of a series of phenomena in which Darwin considered (**10**) that natural selection might operate.

ACKNOWLEDGMENTS.

During the course of many years from 1913 to the present I have been assisted in the present studies (1) on the River Blackwater beds by Dr. J. H. Salter, Messrs. L. French, H. Mole, L. Pearce, J. Pearce, G. French, and Mr. C. Springett ; and (2) on the Whitstable beds by Major A. Gardiner and Mr. E. Luckhurst. The valuable series of temperature observations was obtained in co-operation with the writer by Mr. Laban Pearce from the motor boat *Dan* in all kinds of weather, and by the courtesy of the Tollesbury and Mersea Native Oyster Company. The writer has spent many pleasant days afloat with Mr. Laban Pearce, who has shown special interest and care in obtaining the series of records extending over more than five years. All the work herein recorded prior to September, 1929, was accomplished while the writer was a Naturalist on the staff of the Marine Biological Association, Plymouth ; the work on the River Blackwater was partly subsidised by a Government grant from the Royal Society.

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TABLE VI.

Analysis of Captures of Oyster Drills on the Oyster Beds in the Blackwater Estuary, 1928–30.*

Chart reference.	Designation of bed.	1928 Date.	1928 Uro-salpinx.	1928 Murex.	1928 Pur-pura.	1929 Date.	1929 Uro-salpinx.	1929 Murex.	1929 Pur-pura.	1930 Date.	1930 Uro-salpinx.	1930 Murex.	1930 Pur-pura.
B	Deeps	31/8	44	31	0		N.R.†				N.R.†		
B–A_1	Thornfleet and Deeps			N.R.		15/6	313+*14*	1+*16*	96+*11*		do.		
C_1, D, E	T. Pier, S. Shore, Flat Ground	17/10	46	262	325			N.R.		C_1 23/4	445+*3*	2+*19*	589+*16*
C_1	T. Pier–Nass End	26/10	45	423	563			do.					
C_2–D	Back of Nass and Flat Ground			N.R.		2/6	88+*0*	0+*0*	3+*0*	13/5	692+*8*	0+*20*	192+*27*
$C_{2\text{-}3}$	Back of Nass			do.		6/7	15+*2*	0+*9*	68+*6*				
D	Flat Ground, N. Shore			N.R.				N.R.		29/4	361+*3*	0+*7*	41+*13*
D	N.E. Ground near E. Boundary			do.				do.		19/5	885+*12*	1+*33*	123+*23*
D	Flat Ground, do.			do.				do.		24/5	1875+*15*	3+*47*	258+*31*
	Totals		135	716	888		416+*16*	1+*25*	167+17		4258+*41*	6‡+*126*	1203+*110*
	% living		**7·8**	**41·2**	**51·0**		**71·2**	**0·2**	**28·6**		**77·9**	**0·1**	**22·0**
	Gross total for year			1739				584+*58*				5467+*277*	
A_1	Thornfleet			N.R.		2/7	135+*4*	0+*1*	11+*0*	28/3	15+*0*	0+*0*	0+*0*
A_1	do.			do.		9/7	214+*6*	0+*4*	12+*2*	4/5	30+*0*	0+*0*	0+*0*
A_1	do.			do.						16/6	100+*0*	0+*0*	1+*1*
A_1	do.			do.						19/6	290+*3*	0+*3*	3+*2*
A_2	Mersea Fleet			do.		7/6	115+*0*	0+*0*	0+*0*	20/2	20+*0*	0+*0*	1+*0*
A_2	do.			do.		7/11	107+*1*	0+*0*	6+*0*	20/5	386+*2*	0+*0*	1+*0*
A_3	Salcott Fleet			do.				N.R.		23/5	101+*0*	0+*0*	0+*0*
A_3	do.			do.				do.		13/5	160+*1*	0+*0*	0+*0*
A_4	Tollesbury Fleet			do.				do.		20/2	81+*0*	0+*0*	0+*0*
	do.			do.				do.		13/5	310+*3*	0+*0*	4+*0*
	do.			do.				do.		22/5	424+*0*	0+*0*	4+*0*
	do.			do.				do.		30/5	208+*0*	0+*0*	1+*0*
	do.			do.				do.		6/6	319+*0*	0+*0*	1+*0*
	Totals "A" Grounds			N.R.			571+*11*	0+*5*	29+*2*		2444+*9*	0+*3*	16+*3*
	Totals all Grounds		135	716	888		987+*27*	1+*30*	196+*19*		6702+*50*	6+*129*	1219+*113*
	Gross yearly total			1739				1184+*76*				7927+*292*	

* The numbers in italics refer to dead animals. † N.R. No record. ‡ Individuals of small or medium size.

TABLE VII.

MEAN MONTHLY TEMPERATURE OF THE SEA OVER THE OYSTER BEDS IN THE BLACKWATER ESTUARY FOR THE PERIOD OCTOBER 1925 TO DECEMBER 1930. (From daily observations at about High and Low water (except Sunday), mainly at Thornfleet and Tollesbury Pier Stations (see Fig. 1 and description, p. 305).

	H.W.	No. of Obs.	L.W.	No. of Obs.	Mean of all Obs.	No. of Obs.	H.W.	No. of Obs.	L.W.	No. of Obs.	Mean of all Obs.	No. of Obs.
			1925						1926			
Jan.							38·5	26	37·7	26	38·1	52
Feb.							43·1	24	43·3	24	43·2	48
Mar.							44·6	26	44·5	27	44·5	53
April							49·0	24	49·4	25	49·2	49
May							53·2	24	54·0	24	53·6	48
June							61·2	26	61·7	26	61·5	52
July							66·2	28	65·9	28	66·0	56
Aug.							65·5	26	65·5	27	65·5	53
Sept.							63·8	24	63·8	25	63·8	49
Oct.	52·3	28	51·6	28	52·0	56	52·4	25	51·7	24	52·0	49
Nov.	39·7	23	38·3	23	39·0	46	44·9	26	44·4	26	44·7	52
Dec.	36·6	25	35·4	26	36·0	51	40·0	24	39·0	24	39·5	48
			1927						1928			
Jan.	37·8	24	37·3	23	37·6	47	37·6	25	37·9	26	37·8	52
Feb.	38·8	23	38·5	23	38·6	46	41·8	23	41·3	24	41·5	47
Mar.	44·8	26	45·4	27	45·1	53	42·8	26	43·4	25	43·1	51
April	49·5	25	49·8	26	49·6	51	47·9	24	48·1	25	48·0	49
May	56·9	27	57·0	26	57·0	53	54·3	27	54·6	27	54·5	54
June	59·8	27	59·7	24	59·6	68	60·8	26	61·1	26	60·9	52
July	63·7	24	63·7	25	63·7	79	67·6	25	68·1	26	67·8	55
Aug.	64·6	25	64·6	26	64·8	62	64·4	25	64·0	26	64·2	51
Sept.	59·8	26	59·2	25	59·5	51	61·6	25	60·9	25	61·3	50
Oct.	53·5	24	53·0	24	53·2	48	53·5	26	52·5	26	53·0	52
Nov.	46·3	26	45·9	26	46·1	53	47·5	25	46·1	25	46·8	50
Dec.	37·9	25	36·8	25	37·4	25	39·9	23	38·9	23	39·4	46
			1929						1930			
Jan.	34·4	27	34·0	27	34·2	54	42·0	25	41·9	25	42·0	50
Feb.	32·9	23	32·1	22	32·5	45	38·7	24	38·2	24	38·5	48
Mar.	38·1	23	39·7	26	38·9	49	42·3	25	42·4	25	42·3	50
April	45·4	25	44·7	24	45·1	49	47·8	25	48·1	25	47·9	50
May	54·7	26	55·5	26	55·1	52	55·5	25	56·6	26	56·1	51
June	61·4	25	61·4	25	61·4	50	64·4	25	65·6	24	65·0	49
July	64·8	27	65·2	27	65·0	54	65·2	27	64·9	27	65·0	54
Aug.	64·2	26	63·7	26	64·0	52	62·9	25	63·0	26	62·9	51
Sept.	64·4	23	64·3	23	64·4	46	62·3	24	61·4	25	61·8	49
Oct.	54·4	27	53·2	27	53·8	54	53·5	27	52·8	27	53·2	54
Nov.	45·4	24	44·7	26	45·0	50	45·4	25	44·7	25	45·0	50
Dec.	42·9	23	42·1	22	42·5	45	42·0	23	41·4	22	41·9	45

Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part II. On the Gonad of Egg-Spawning Individuals.

By

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and

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With 12 Figures in the Text.

In an earlier publication (1, 1927) it has been shown that female-functioning oysters (*O. edulis*) normally begin to develop spermatozoa with great rapidity immediately after extruding a batch of eggs. Thus, while the extruded eggs are developing in the mantle cavity of the oyster, spermatozoa are developing at the same time within the body of the oyster. In these circumstances sperm develop so quickly that a few hours after the extrusion of eggs small clumps of characteristic spermatocytes can be detected in the gonad either in the fresh material or in microscopic sections. Within about 14 hours from the extrusion of ova, relatively large clumps of spermatocytes are easily recognisable in preparations of the fresh gonad ; while within about 45 hours the gonadial tubes become filled with maturing sperm, and in some cases sperm-morulæ may already occur. After $3\frac{1}{2}$ days from egg-spawning the percentage of gonads containing ripe sperm rapidly increases, until at about $8\frac{1}{2}$ days after the extrusion of ova when the larvæ which the oyster is carrying have become fully developed veligers ready to be extruded—sperm development may have proceeded so far that the gonadial ducts themselves become filled with ripe sperm-morulæ as shown in Figure 1, p. 316. The rates of development quoted above are averages for those occurring in English oyster beds ; but are with little doubt mainly dependent on the prevailing temperature.

After an oyster has extruded its larvæ sperm-production continues for a few weeks, but begins to wane about one month after egg-spawning (see **1**, Table XII, p. 1035, 1927). Two months after the last egg-spawning (if this has occurred in early- or mid-summer) sperm-production has usually ceased ; the gonad passes into a quiescent condition (in British waters and

under normal conditions) and a period of "fattening," or the accumulation of reserves, usually follows. If egg-spawning occurs late in the summer, the sperm subsequently developed may be retained over the winter period. A full discussion of such cases will, however, be given later when data can be presented.

In this paper figures are given to illustrate successive stages of the gonad after egg-spawning, and also the state of the ripe female gonad in the spawning condition. As the condition of the gonad can usually be easily and quickly ascertained by an examination of a fresh preparation of the gonad, figures of the microscopic fields obtained from fresh preparations

Photo. A.J.S.

FIG. 1.—View of the visceral mass of a recently black-sick oyster with gonoducts already filled with ripe sperm-morulæ.

(Photo of the living animal by Mr. A. J. Smith.)

are given as well as those of microscopic sections. In order to obtain a fresh preparation of the gonad of the oyster a cut may be made in the visceral mass near the pericardium or dorsally near the loop of the intestine which encircles the stomach (see **2**, Plate XII). In this way is avoided a possible incision of the main gonoducts which lie mostly superficially. When it is obvious that the gonoducts are empty, the visceral mass may be cut across entirely. When the cut is made a microscope slide is pressed gently against the cut in order to collect a little of the fluid which is exuded. This drop of fluid is examined at once under the microscope without a cover-slip, and without adding sea-water; it is advisable to try several samples until familiarity with the method is obtained. To test the ripeness of the sperm, sea-water may afterwards be added, when—if the preparation has not been allowed to dry—it will be found that ripe sperm-morulæ break up into separate active sperm on the slide.

In Table I, p. 318, are given full details of the series of figures shown herein with the correlated states of development of both the embryos (or larvæ) carried by an oyster, and the sperm-morulæ within the body of the same individual.

The arbitrary periods, A, B, C–F, are defined in **1**, p. 983, and the arbitrary categories I, III–X, are shown in **1**, Table II, p. 981 ; arbitrary periods G to J are defined *loc. cit.* on p. 1022. The significance of the correlation between the arbitrary period and the arbitrary categories associated in Table I herein can be seen at a glance in **1**, Table XII, p. 1035.

Acknowledgments.

One of us (C. A.) takes this opportunity to thank the Council of the Marine Biological Association for the kind hospitality extended to him to work in its laboratory ; and to express his gratitude to Dr. E. J. Allen and Professor D. M. S. Watson for their encouragement and help.

Our thanks are due to Mr. A. J. Smith for the photograph for Fig. 1.

SUMMARY.

Illustrations are given of successive stages of the gonadial products of the European oyster (*O. edulis*) after the act of egg-spawning. Both microscopic and fresh preparations are figured.

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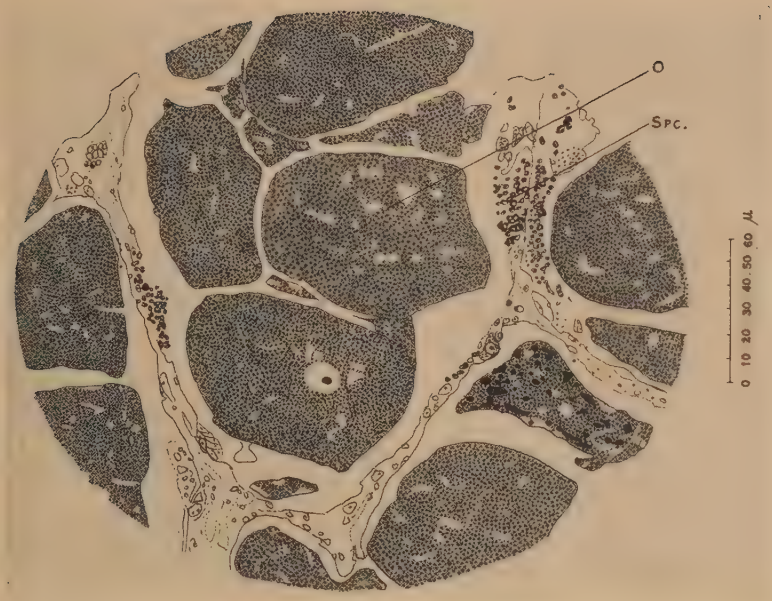
1. Orton, J. H. 1927. Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part I. The Change from Female to Male. Journ. Mar. Biol. Assoc., N.S., Vol. XIV, No. 4, pp. 967–1045.

2. Orton, J. H. 1924. An Account of Investigations into the Cause or Causes of the Unusual Mortality among Oysters in English Oyster Beds during 1920 and 1921. Part I. Fish. Invest., London, Ser. II, Vol. VI, No. 3, 1923 (1924).

TABLE I.

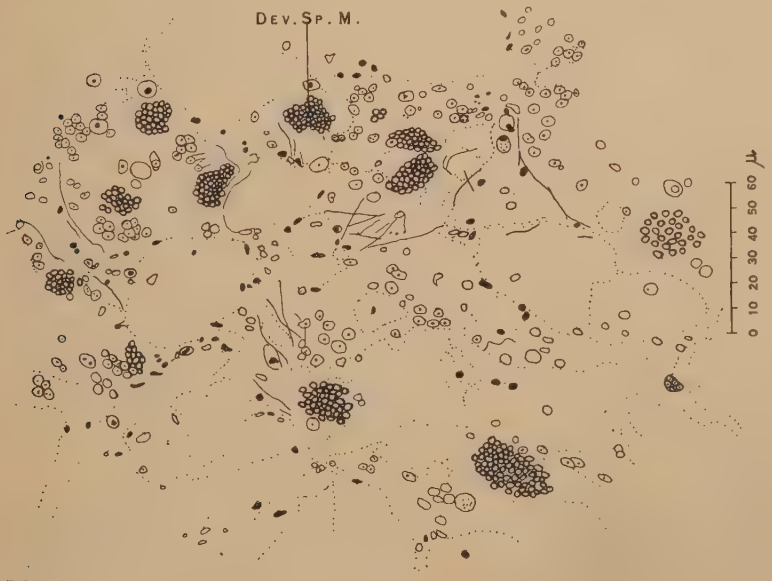
CORRELATED STATES OF DEVELOPMENT OF SPERM AND EMBRYO IN INDIVIDUALS OF *O. EDULIS*, ILLUSTRATED HEREIN.

	Condition of eggs, embryos or larvæ.	Approx. age of embryos or larvæ.	Date and locality.	Arbitrary period.	Sex-cells in gonad. sperm-morulæ ripe.	sperm-morulæ unripe.	ripe ova.	Arbitrary category.	Description of illustration.
Fig. 2	Ripe eggs; from female in act of spawning.	0 hours	22 June, 1926 Truro beds	A	none	none	∞	I	Section of gonad.
Fig. 3	1 and 2-celled embryos	2 ,,	31 May, 1927 West Mersea	A	none	fair no. very young	few spots	III	Section of gonad microscopic field.
Fig. 4	1 and 2-celled with 2 to 5 nuclei	2½ ,,	30 Aug., 1929 R. Yealm	A	none	few up to 40μ	∞ in patches	III	From living gonad microscopic field.
Fig. 5	10% in 32-celled stages	20 ,,	14 Oct., 1927 Truro beds	B	none	fair no. up to 60μ	f. ∞ in spots	IV	From living gonad.
Fig. 6	about 64-celled stages	46 ,,	16 Sept., 1929 R. Yealm	C	none	∞ ca. 60 μ	none	IV	Microscopic field from living gonad.
Fig. 7	Ciliated embryos with trace of shell	3 days	1 June, 1927 West Mersea	D	none	∞ large nearly ripe	rare	IV	Section of gonad.
Fig. 8	White-sick; larval shell about 100μ	4½ ,,	31 July, 1930 R. Yealm	E	few to fair no.	∞	rare	V	Microscopic field from living gonad.
Fig. 9	Black-sick; fully shelled larvæ	6–8 days	8 Oct., 1926 Truro beds	F	∞	∞	none	VI	Section of gonad.
Fig. 10	Black-sick; larval shell 200μ	,,	25 July, 1929 R. Yealm	F	∞	∞	none	VI	Microscopic field from living gonad.
Fig. 11	Post-sick oyster	estimated 1–2 months	16 Sept., 1929 R. Yealm	H or I	∞	none	none	VIII	Microscopic field from living gonad.
Fig. 12	Post-sick oyster	ca. 50 days	10 Sept., 1930 R. Yealm Spawned 31.7.30	H	few or fair no.	none	none	IX	Microscopic field from living gonad.



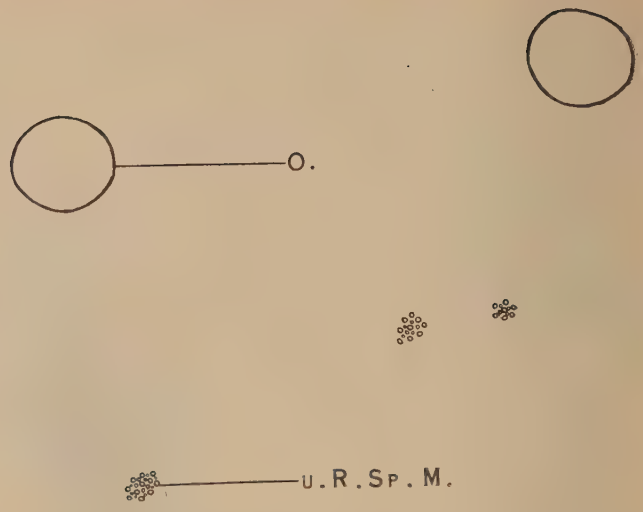
Del. C.A.

Fig. 2.—Microscopic section of the gonad of an individual of *O. edulis* caught in the act of spawning, 22 June, 1926. O. mature ova filling the gonadial tubules. Spc. gametogonia destined to become spermatocytes.



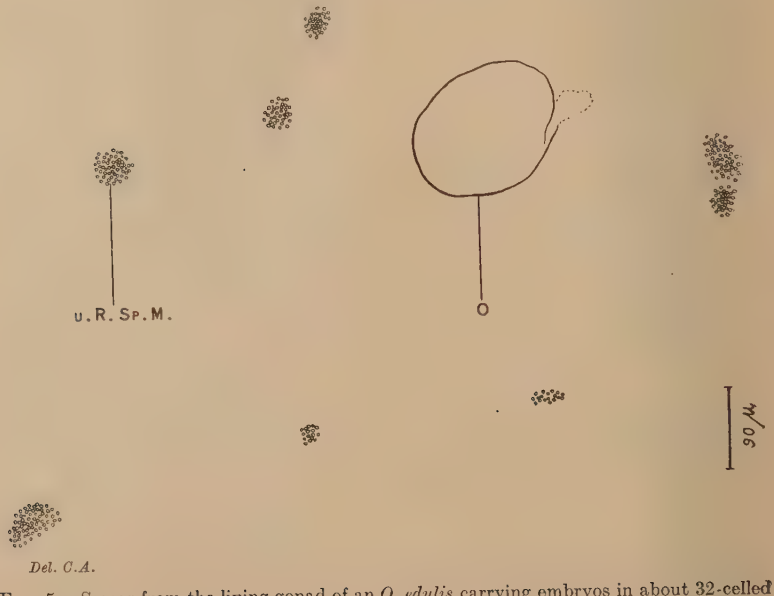
Del. C.A.

Fig. 3.—Microscopic section of gonadial tubules of *O. edulis* carrying 1 to 2-celled embryos showing very young developing sperm-morulæ. (Dev. Sp. M.)



Del. C.A.

FIG. 4.—Smear from the living gonad of an *O. edulis* carrying 1 to 2-celled embryos, showing unspent ova, O (about 150μ in diameter), and small developing sperm-morulæ.

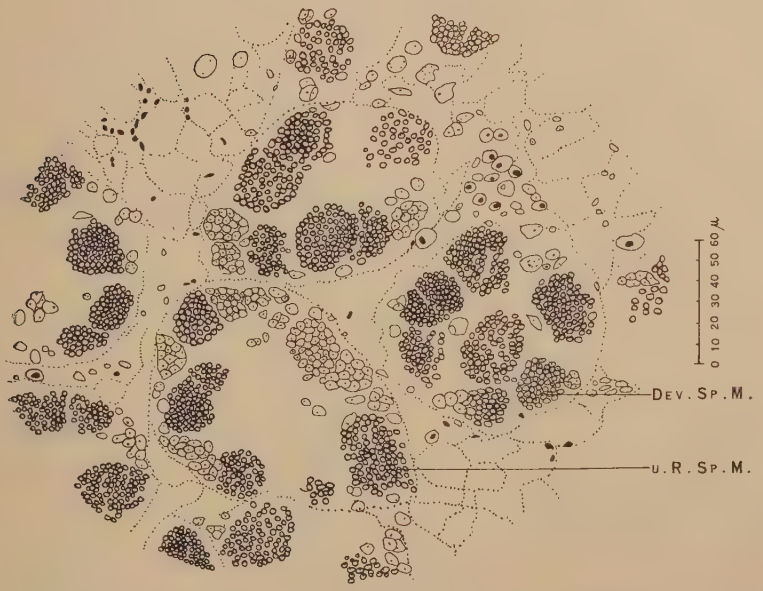


Del. C.A.

FIG. 5.—Smear from the living gonad of an *O. edulis* carrying embryos in about 32-celled stages, showing unspent ova, O, and fairly numerous developing sperm-morulæ (u.R.Sp.M.) about 60μ long.

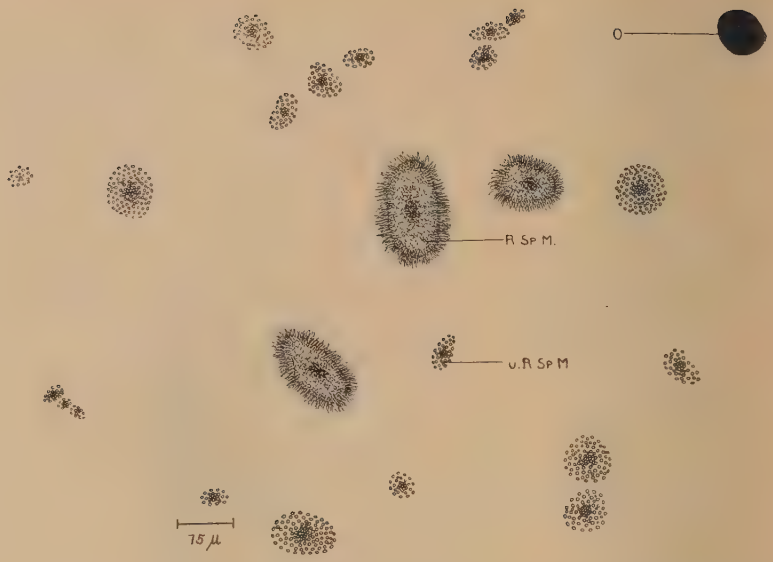
Del. C.A.

FIG. 6.—Smear from the living gonad of an *O. edulis* carrying 64-celled embryos, showing numerous large developing sperm-morulæ (u.R.Sp.M.) about 60μ long.



Del. C.A.

FIG. 7.—Microscopic section of gonad of *O. edulis* carrying embryos which are ciliated and show a trace of the developing shell, showing numerous large and almost ripe sperm-morulæ (u.R.Sp.M. and Dev.Sp.M.).

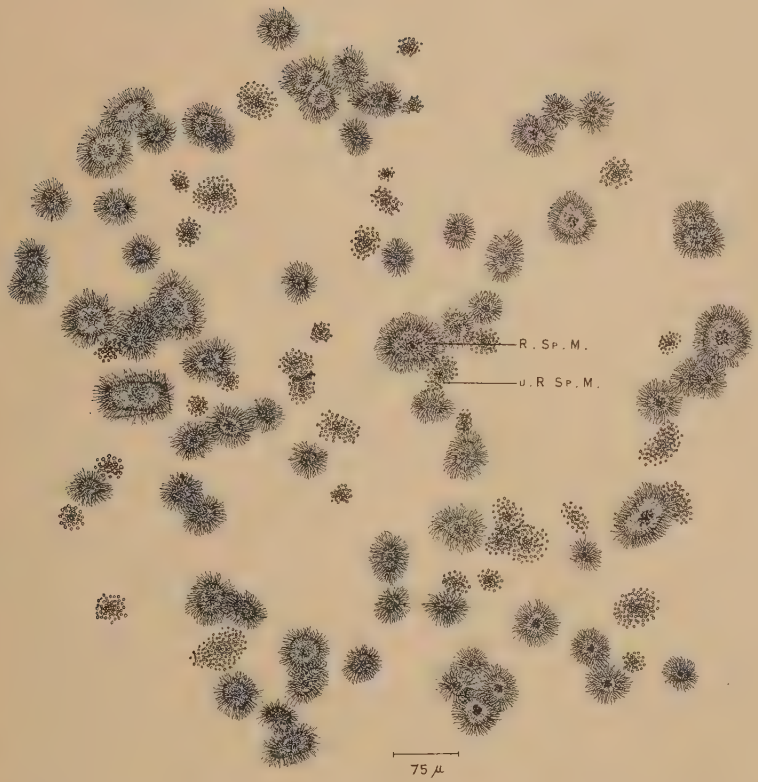


Del. C.A.

FIG. 8.—Smear from the living gonad of a grey-sick *O. edulis*, i.e. carrying embryos with half-developed shells and no pigment in the digestive organ, showing numerous large developing sperm-morulæ (u.R.Sp.M.), a fair number of ripe sperm-morulæ (R.Sp.M.), and a degenerating ovum, O.

Del. C.A.

FIG. 9.—Microscopic section of gonadial tubule of a black-sick oyster carrying fully-developed larvæ (i.e. shells 200μ long) and showing ripe (R.Sp.M.) and large developing sperm-morulæ (u.R.Sp.M.), and also younger masses (Dev.Sp.M.) *in situ*.



Del. C.A.

Fig. 10.—Smear from the living gonad of a black-sick oyster, showing numerous ripe (R.Sp.M.) and numerous large unripe sperm-morulæ (u.R.Sp.M.) in a small portion of the microscopic field.

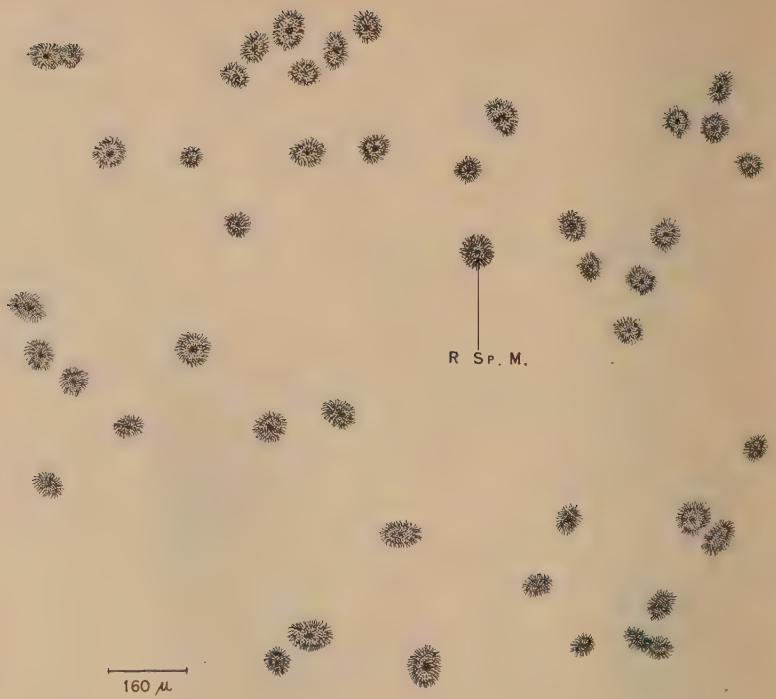


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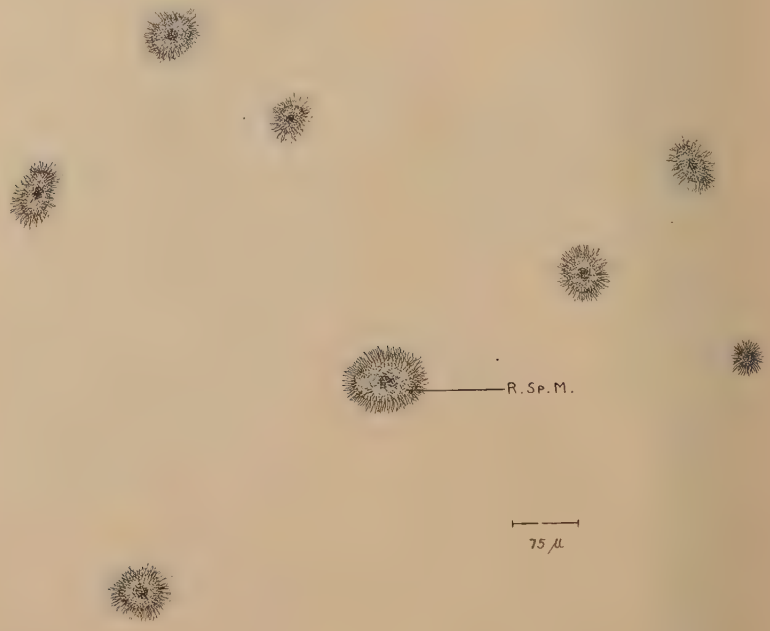
FIG. 11.—Smear from the living gonad of a post-sick *O. edulis* (identified from seasonal studies in sex-change) showing numerous ripe sperm-morulæ only.



Del. C.A.

FIG. 12.—Smear from the living gonad of a post-sick *O. edulis*, showing a fair number of ripe sperm-morulæ only. (White-sick 31 July, 1930 ; kept in tanks afterwards at Plymouth until 10 September, 1930.)

The Muds of the Clyde Sea Area. III. Chemical and Physical Conditions; Rate and Nature of Sedimentation; and Fauna.

By

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With 11 Figures in the Text.

A PRELIMINARY survey of the area with regard to the distribution of the total nitrogen and phosphate content of the muds was described in a previous paper (Moore, **5**). A number of stations were worked, and these were further examined for nature and distribution of particles, water content, and density, and as a result of this survey certain stations were chosen as suitable for more extensive work. At the same time it became evident that the layers in which the greatest and most important changes were taking place were those within a few centimetres of the surface, and these were therefore studied most intensively.

Among these stations many show very irregular deposition, owing to tidal currents or other causes. At some, conditions appear to be comparatively stable, and a series of these was chosen to illustrate the four chief types of mud found in this area, which are as follows :—

Deep Water Type. This is found in the deepest channels where there is little or no tidal action, a slow rate of sedimentation and very little influence from the land, e.g. Station 7, 110 metres ; St. 7b, 166 metres ; St. 23, 73 metres.

Mid-Loch Type. This is a shallower water type than the preceding, usually in a depth of 45 to 75 metres. There is little tidal action and the small amount of matter received from streams is of very fine grade. Deposition is more rapid, and the mud tends to be softer than in the previous type, e.g. Station 8b, 37 m. ; St. 9, 37 m., Kyles of Bute ; St. 11, 73 m., Loch Striven ; St. 13, 73 m., Loch Goil ; St. 13a, 82 m. ; St. 15, 58 m. ; St. 15a, 64 m., Loch Long ; St. 16a, 26 m., Holy Loch.

Loch Head Type. Here there is practically no tidal action ; the depth is from 20 to 25 metres, with the exception of Loch Goil Head where it is deeper. Only small streams enter the lochs, but their influence is most marked on these loch-head stations. The mud is of a more open composition, containing more numerous and larger vegetable remains and sand

grains, e.g. St. 10, 24 m., Loch Striven ; St. 12, 51 m., Loch Goil ; St. 14, 22 m., Loch Long ; St. 16, 22 m., Holy Loch ; St. 18, 24 m., Gare Loch.

Sandy Mud Type. Although this work has not been extended to shore sands, some sandy muds have been examined for comparison. These occur chiefly in bays and in parts of channels where there is evidence of strong tidal action, e.g. St. 1b, 25 m., Kames Bay, Cumbrae ; St. 17a, 73 m., Dunoon Basin ; St. 25, 46 m., Barrier Plateau.

A number of stations not described in the previous paper have since been worked and their positions are defined in Table I.

TABLE I.

Positions of New Stations.*

St. 1b.	Kames Bay, Cumbrae, 25 m.
St. 1c.	100 yards North of Hunterston Perch, Fairlie Sands, 22 m.
St. 7b.	3·5 miles West of Garroch Head, 166 m.
St. 17d.	Kilchattan Bay, 22 m.
St. 23.	South of Little Cumbrae light, West of Ardrossan, 73 m.
St. 25.	Midway between Ailsa Craig and Campbelton, 46 m.

Method of Sampling.

The samples consisted of undisturbed cores of mud 3·2 cm. in diameter and up to 40 cm. in length. The sampler (Moore and Neill, **6**) collects the cores in glass tubes which are then corked at both ends and taken to the laboratory for examination.

Soluble Salts.

Under natural conditions the soluble salts leave the mud partly by diffusion into the overlying water via the interstitial water of the mud itself, and partly also by the expression of this interstitial water during the packing of the mud. In these estimations, therefore, wet mud was shaken up with a large amount of sea-water, and the increase in concentration of the various salts in this determined ; in the case of phosphate, it being undesirable to extract with a citric acid solution, a sample of the mud which when dry would weigh about 100 gm. was rubbed down in a little sea-water until no lumps remained, and was then shaken with 2200 c.c. of sea-water for two hours in an end-over-end shaker. This was then allowed to stand for 24 hours in the dark, and the supernatant water siphoned off for analysis. Shaking for this period was found to remove all the soluble phosphate. The results for the other soluble salts are not yet completed.

* The localities of all other stations referred to are given in Journ. Mar. Biol. Assoc., N.S., Vol. XVI, No. 2, 1930, p. 597.

For the estimation of soluble phosphates, Atkins' modification of Denigès method (Atkins, **1**) was used, the water being passed through a washed Whatman No. 3 filter paper before the estimation. This was found to give identical results with samples cleared by centrifuging.

The results for soluble phosphates are given in Table II, and graphically in Figure 1. St. 11 is in most respects normal, so that the curve shown is

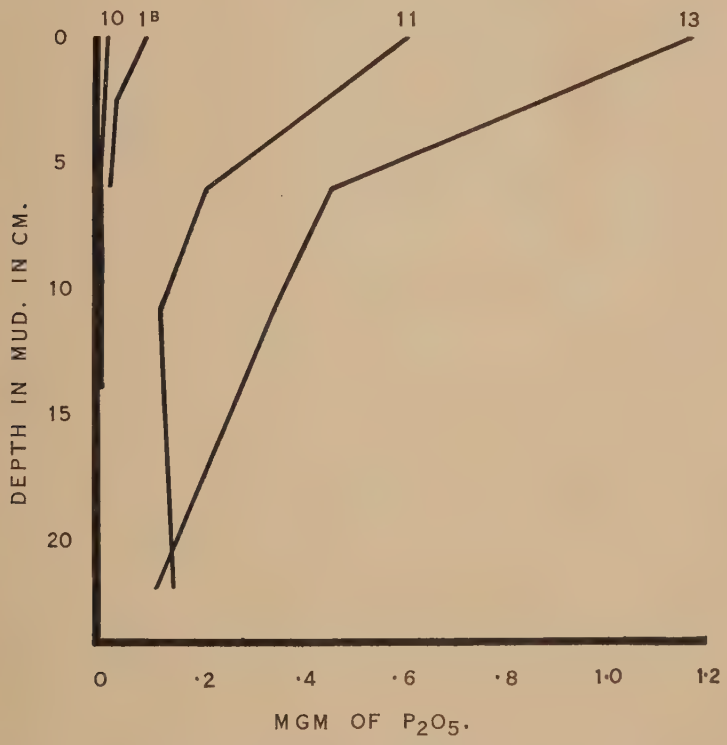

Fig. 1.—Distribution of Soluble Phosphate with depth in the mud.

probably typical for a mid-loch station. It agrees in shape with that for St. 13, except that the latter is much richer in soluble phosphate as it is in other salts including total phosphate. In both, the values are high at the surface of the mud but decrease rapidly to a depth of 6 cm., and then much more slowly. The very low values at St. 1b are to be expected in a sandy mud, but the even lower values at St. 10, at Loch Striven Head, are unexpected as the total phosphate there is almost as high as that at St. 11. The more open condition of the mud may allow more rapid loss

by diffusion, but this does not seem to be sufficient to account for the great difference between the two stations.

It is significant that the greatest loss of soluble phosphate occurs in the same layer as that from which the greatest amount of interstitial water is being expressed by packing, that is the upper 6 cm.

Oxygen Absorption by Mud.

The condition of the mud with regard to oxygen was estimated by shaking samples of the mud with sea-water of known oxygen content, and measuring the decrease in the latter by Winkler's method. From the

TABLE II.

Soluble P_2O_5 in mg. per 100 gm. of mud.

Station.	Depth.	Sol. P_2O_5.
1b	0–1 cm.	0·08
	2–3	0·03
	5–6	0·02
10	0–2 cm.	0·01
	4–6	0·004
	8–10	0·006
	12–14	0·006
11	0–2 cm.	0·54
	5–7	0·21
	10–12	0·12
	20–22	0·14
13	0–2 cm.	1·05
	5–7	0·46
	10–12	0·34
	20–22	0·13

amount taken up in this way it is clear that not only can there be no dissolved oxygen in the interstitial water even at the extreme surface in any mud examined, but also that any oxygen diffusing in from the overlying water will be rapidly absorbed. The general results for all types of station show an increase in the amount of the immediate chemical absorption, as distinct from the slower biological oxygen absorption down to a depth of about 5 cm., below which it becomes more or less constant at about 0·3 to 0·5 mg. of oxygen absorbed per gm. of mud (dry weight). In the mid-loch stations where there is a rain of rich organic matter on the surface, the amount of oxygen absorbed by this surface

layer is correspondingly high, but in the loch head and sandy types the mud is of a more open consistency, allowing deeper diffusion of the overlying water, and this phenomenon is not shown.

Reaction.

The estimation of the pH of the interstitial water of the mud presents certain difficulties; it is not usually possible to withdraw such water except in small quantities and only by the use of reduced pressure on a filter, which would affect the CO_2 content and hence the pH. Also turbid solutions may be obtained, so that a colorimetric method of estimation is not reliable.

For the latter reason a quinhydrone electrode was used, with a calomel half-cell as standard (Clark, **2**). The soil analysis method of shaking the dried soil with distilled water, is not applicable here, and even shaking the fresh wet mud with distilled water yields erratic results, and always of a low value. On the other hand, if the mud is shaken with sea-water, or with saline solution, regular values are obtained, and these are in agreement with the values obtained from the interstitial water itself in those cases where the mud is of such consistency as to allow of the easy withdrawal of the latter. In practice a tube of mud was cut up into one-centimetre layers, and each of these immediately shaken up with about 20 c.c. of sea-water, the buffering power of the mud being sufficient to overcome that of the water. Quinhydrone was then added to this and the pH read as quickly as possible. The method is in agreement with that recommended by the International Society of Soil Science (**3**) with the exception that their suggestion is the use of a solution of KCl where we have used NaCl. The use of quinhydrone in the presence of a reducing medium such as a mud extract necessarily introduces an error, but the rate of potential drift suggests that this should be small if the estimation is made quickly after the addition of the quinhydrone.

The results for the four types of station are shown in Figure 2. In general, with increasing depth in the mud there is a steady rise in pH followed by a less rapid fall again, the depth at which the maximum occurs varying from one station to another. The overlying water has a higher pH than the mud, and its influence seems to affect the top centimetre layer of the mud whose pH is usually distinctly higher than that of the succeeding centimetre. In the case of the more open sandy and loch-head types, this influence is also felt in the second centimetre. The absence of the rise in the mud from St. 13 is probably due to experimental error, it being very difficult to isolate the semi-fluid surface layer in this case. The drop at 5 to 6 cm. at St. 11 in Loch Striven is a peculiarity of that loch [*vide* distribution of total P_2O_5 (Moore, **5**)], as it occurs in most parts of the channel but not in other lochs.

Considering the high organic content of these muds it is surprising that the pH values are so high, the lowest recorded being only 7·45. Curiously, too, a mud as rich as that at St. 13 may have as high a pH as 7·84 well

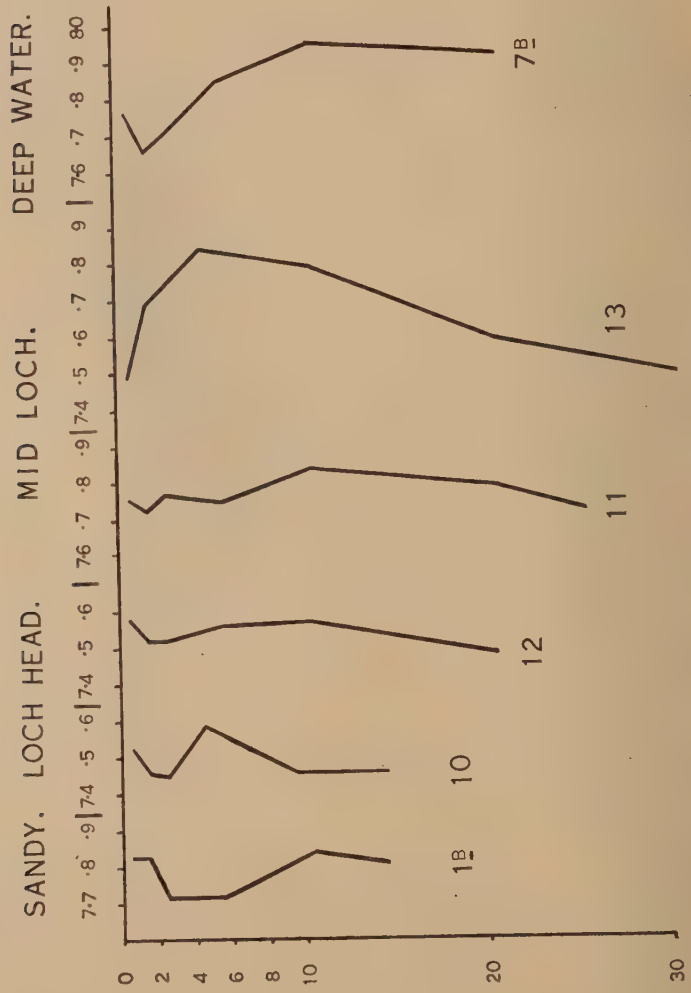

Fig. 2.—Distribution of pH with depth in mud (ordinate) in cm., for the four types of station.

below the surface. The higher surface values are probably to be explained by the influence of the sea-water which has always a higher value than that of the mud. The rise in pH from 2 cm. downwards may be due to the decreasing amount of organic matter with depth, but this gives no account

of the succeeding drop which is so marked in the deepest samples taken, i.e. St. 11 and 13. From a biological aspect, however, the outstanding point is the absence of any extremes of pH even in very different types of mud. The actual values obtained are shown in Table III.

TABLE III.

Distribution of pH with Depth in Mud.

Depth in. Mud in cm.	Stations. 1b.	10.	12.	11.	13.	7b.
0–1	7·83	7·53	7·58	7·76	7·49	7·76
1–2	7·83	7·46	7·52	7·73	7·69	7·66
2–3	7·72	7·45	7·52	7·77	—	7·70
4–5	—	7·59	—	—	7·84	—
5–6	7·72	—	7·56	7·75	—	7·85
9–10	—	7·46	—	—	—	—
10–11	7·84	—	7·57	7·84	7·79	7·95
14–15	7·81	7·46	—	—	—	—
20–21	—	—	7·48	7·79	7·59	7·92
25–26	—	—	—	7·72	—	—
30–31	—	—	—	—	7·49	—

Water Content.

The distribution of density and water content with depth in the mud was estimated simultaneously in a number of muds. The method found to be most applicable was that of weighing about 10 gm. of the wet mud, on a tared watch-glass, then reweighing immersed in sea-water, and finally tipping off the free water from the glass, drying in a water oven at 100° C., and weighing again. From these results the density of the wet and of the dried mud, and the percentage by volume of water in the wet mud can be calculated. The chief error introduced is that of the salt content of the interstitial water, and of such water as clings to the mud and the watch-glass on drying. This will cause the figures for density to be about 5% too high, but since the water content does not vary sufficiently to affect this appreciably, the error has been assumed to be constant, and no correction has been made for it. Similarly, the figure for the percentage of contained water will be about 2% too low and will also have an almost constant error. The error due to weighing in sea-water instead of fresh is allowed for.

The values obtained for the various stations, which are expressed as percentages by volume of water in the wet mud, are given in Table IV. It must be understood that these figures do not refer to the amount of true free interstitial water, but to the total loss on drying at 100° C., some of

TABLE IV.

Distribution of Water Content with Depth in Mud.

Values expressed as percentage of water, by volume, in the wet mud.

Station.	Depth in mud in cm. 0–2·5.	2·5–5.	5–7·5.	7·5–10.	10–12·5.	12·5–15.	15–17·5.	17·5–20.	20–22·5.	22·5–25.	25–27·5.	27·5–30.	30–32·5.
1	74·4	69·4	70·9	70·2	67·7	66·6							
11	84·6	81·0	78·3	78·8	77·7		77·5	78·6	77·9	76·8	77·6	76·0	
11b	80·8	80·1	78·6	78·4	77·3	77·3	77·8	76·6	74·9	72·9	72·9	72·1	68·4
12	81·0	81·7	78·9	79·1	77·6	77·7		74·0	72·8	75·7	75·7		
13	86·7	87·1	85·0	85·2	81·7	81·2	83·5		80·2	80·1	80·8	79·0	81·0
13a	80·4	81·7	80·7	79·7	78·2	79·1	77·9	79·1	79·1	74·7			
14	82·4	78·9	78·8	78·3	77·4	77·6	77·7	76·9	77·8	75·2		76·3	
15a	81·4	79·6	79·8	78·5	76·1	74·3	76·2	76·1	75·6	76·2	74·4	74·6	
16	79·3	77·2	75·1	74·0	72·5	71·4	68·3	71·3	72·2				
16a	77·7	73·8	73·0	73·7	72·2	71·2	71·1	70·1	70·2	69·1	65·6	64·9	
17	81·5	79·8	77·3	77·7	76·8	74·8	76·0	77·2	73·5	75·3			
17a	59·6	60·8	58·1	60·4	54·4	48·0							
17b	80·9	77·5	77·5	76·3	77·1	76·3	80·7	77·3	76·7	75·0	74·8		75·4
17c	74·5	72·6	75·2		71·5	73·4	72·6	70·7	71·5	68·6	68·5		
19		81·2		77·4		75·5		74·3		74·2			
20a	50·7	56·6	68·1	71·2	71·3	71·4	66·9	71·5	55·5				
23		80·1		74·7		76·2		73·9		73·9		71·9	
24	82·8	80·3	78·7	77·7	76·7	73·6	76·1	74·3	74·2	72·4	72·9	68·6	
26	71·7	68·9	68·1	66·8	64·5	62·8	58·8	59·4	56·3				

which may be due to water contained in the form of gels or otherwise fixed.

Figure 3 shows the distribution of the water content with depth in the mud for St. 11, a mid-loch type. Here there is a rapid fall in the quantity of water from 85% at the surface to 79% at about 6 cm. Below this depth the fall is very much slower. Though the slope of the curve varies at different stations, there occurs typically a rapid fall in water content near the surface followed at a varying depth by a more slowly decreasing or constant amount. The depth at which this constant amount is attained

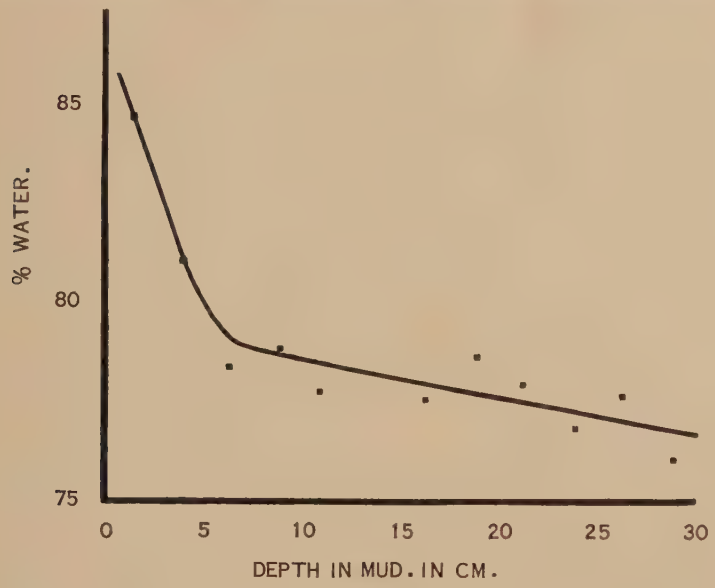

Fig. 3.—Variation of water content with depth in mud at St. 11—mid-loch type.

varies, and in some cases, i.e. St. 26, the sampling seems not to have been taken deep enough to reach this level, though it would probably be reached with a longer sample. Certain stations are very erratic, notably those in the Dunoon Basin, and of these St. 20a is of interest since a sample there shows at the surface a layer of sand, superimposed at a depth of about 10 cm. on a layer of mud and this again at about 20 cm. on another layer of sand. The water content shows corresponding changes being lower in the sandy layers than in the mud. Since this station must be largely influenced by the river, it would appear that the nature of the material deposited had changed considerably from time to time.

There does not appear to be any correlation between the shape of the curve and the type of station, with the exception of the sandy type which

has a very much lower water content than the softer muds. In the other types the surface values are remarkably high, frequently lying between 80% and 85%. The constant value attained usually lies between 70% and 80%, though in the case of St. 26 the value has fallen from 72% to 56% without becoming constant. This station, however, is far from land and of a different type from that of the lochs.

In a later section it is shown that the mud is laid down on the surface

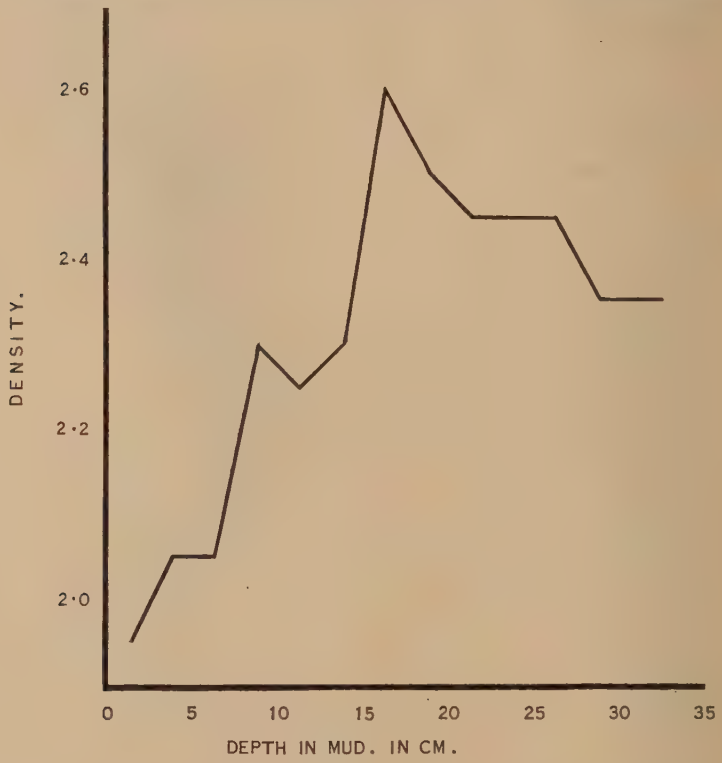

Fig. 4.—Distribution of density with depth in the mud for St. 13—mid-loch type.

in a very fine and loose condition ; and an examination of a tube sample shows that it becomes steadily harder and more like a clay with increasing depth. The above figures show that this is due to packing accompanied by expression of water. The interesting point, however, is that this packing continues for such a long time ; in the case of St. 11, as shown later, the chief packing being completed at the end of about ten years, and at a depth of about 5 cm., while in cases such as St. 26 packing probably continues for a very much longer period.

Finally, the cause of the packing may be assigned to several factors. Primarily, no doubt, it is due to the weight of the superimposed mud, but another factor of importance will be the packing effect of the small organisms, such as worms, moving about in the mud ; while yet another factor of great importance is the consolidation of the fine mud particles into firm pellets in the guts of animals which eat them. This question of pellet formation, which is of tremendous importance in some muds, is discussed more fully later. Finally, there is the possibility of packing accompanying chemical change in the break down of organic matter in the mud.

Density.

The distribution of the density of the dried mud throughout the area is given in Table V, while that for St. 13 is shown graphically in Figure 4, this being fairly typical for the area. Of the nineteen stations worked there was a rise in density below the surface in twelve, followed by a fall in density in half of these. While the density of individual particles may vary much more widely, that of the mud *en masse* lies usually between 2·0 and 2·5. These values, together with an increasing density at greater depths, are to be expected in a mud consisting chiefly of a mixture of quartz and calcareous particles with a varying amount of lighter organic matter, the quantity of the latter decreasing with depth.

The subsequent fall at some stations is less clear, especially as it occurs only in the mid-loch type of station where conditions are stable. Of the loch-head type, three stations show a rise but no fall, possibly because the sampling has not been deep enough, the rate of deposition being greater in these. The stations in the Dunoon Basin—Stations 20a, 17, 17a, 17b, 17c—are usually erratic in every way, St. 20a reflecting its alternate layers of mud and sand in its density. In the sandy type of station there is very little variation, the density being that of the quartz of which it is almost entirely composed.

Nature and Distribution of Particles.

The abundance of fæcal pellets and the large quantity of organic matter present render sieving an unsuitable method of grading ; for the same reason the standard method of grading by rate of settling in distilled water after first drying, treating with H_2O_2 and washing with distilled water, would completely alter the nature of the mud from a biological standpoint. An attempt to estimate particle size from rate of settling of fresh mud in sea-water would also yield erroneous results, since the coarser grades may consist of a mixture of quartz grains with pellets of twice their size, while in the finer grades it has been shown that the density of the particles may vary in extreme cases from 1·2 to over 4·0.

TABLE V.

Distribution of Density with Depth in Mud.

Station	Depth in mud in cm. 0–2·5.	2·5–5.	5–7·5.	7·5–10.	10–12·5.	12·5–15.	15–17·5.	17·5–20.	20–22·5.	22·5–25.	25–27·5.	27·5–30.	30–32·5.
1	2·60	2·60	2·60	2·60	2·60	2·65	—	—	—	—	—	—	—
11	2·30	2·35	2·35	2·40	2·30	2·35	2·40	2·50	2·35	2·30	2·35	2·30	—
11b	2·35	2·45	2·50	2·50	2·45	2·45	2·60	2·60	2·65	2·55	2·55	2·50	2·40
12	2·00	2·20	2·25	2·30	2·30	2·35	—	2·20	2·25	2·30	2·25	—	—
13	1·95	2·05	2·05	2·30	2·25	2·30	2·60	2·50	2·45	2·45	2·45	2·35	2·35
13a	2·30	2·20	2·40	2·45	2·45	2·45	2·40	2·50	2·50	2·25	—	—	—
14	2·25	2·35	2·35	2·55	2·30	2·35	2·40	2·40	2·45	2·35	2·30	2·40	—
15a	2·40	2·45	2·50	2·45	2·45	2·50	2·55	2·60	2·50	2·55	2·60	2·55	—
16	2·35	2·40	2·45	2·40	2·45	2·40	2·35	2·50	2·55	—	—	—	—
16a	2·40	2·45	2·45	2·50	2·50	2·55	2·55	2·55	2·55	2·55	2·60	2·60	—
17	2·50	2·45	2·40	2·45	2·40	2·50	2·40	2·45	2·45	2·45	—	—	—
17a	2·65	2·60	2·65	2·65	2·65	2·65	—	—	—	—	—	—	—
17b	2·50	2·50	2·50	2·45	2·45	2·50	2·65	2·60	2·70	2·55	2·55	2·55	2·60
17c	2·40	2·35	2·45	2·35	2·40	2·40	2·45	2·35	2·45	2·45	2·45	—	—
19	2·30		2·35		2·35		2·40		2·30				
20a	2·50	2·40	2·45	2·40	2·40	2·40	2·40	2·45	2·55	—	—	—	—
23	2·35		2·45		2·45		2·45		2·50		2·50		
24	2·40	2·35	2·35	2·45	2·45	2·45	2·50	2·55	2·50	2·45	2·50	2·55	—
26	2·40	2·45	2·50	2·45	2·50	2·55	2·55	2·60	2·55	2·60	—	—	—

One of the most significant factors in these muds is the conversion into fæcal pellets of the very fine material falling on the surface. In extreme cases the whole of the mud may consist of pellets with no admixture of fine material at all, and it is normal for them to form up to 40% of the total. For these reasons a simple type of elutriator has been used which differentiates the mud into four grades of fairly definite constitution, and gives an idea of the distribution of the pellets and their relation to the fine material. The elutriation is carried out with sea-water, in four upright conical glass vessels connected in series, the vertical rate of flow at the widest part in each being as follows :—

Grade.	Rate of flow in cm. per minute.
G. 1	10·0
G. 2	2·5
G. 3	1·5
G. 4	1·0

The constitution of these grades is fairly constant and the following description of the grades from a bucket sample from St. 10 may be generally applied. Where there are many fragments of plant origin they are distributed in all grades according to size. Occasionally also in the surface layers Grade I may contain numerous very small worm tubes.

Grade I. Chiefly pellets of large Maldanid worms (length 0·2 to 1·0 mm.) and of *Syndosmya alba* (length ca. 0·5 mm.); also some large masses of organic detritus, and sand grains of about 0·1 mm. long. In deep-water stations there are very few large sand grains.

Grade II. Almost entirely pellets of Maldanids (length up to 0·2 mm.), and some small sand grains.

Grade III. Chiefly small mineral fragments, with masses of fine detritus, some recognisably of organic origin, up to 0·1 mm. long, and a few small Maldanid pellets of the same length or less.

Grade IV. Similar to Grade III only of smaller size and with no pellets. The small amount of material passing Grade IV has been neglected.

Before elutriation it is necessary to rub the mud in some sea-water into a fluid condition. This causes an appreciable amount of break down of the large pellets in Grade I. The surface mud is soft and needs very little rubbing, so that in it there will be little break down, but in the deeper and harder muds more rubbing is necessary, and hence more break down takes place. The pellets in Grade II are smaller, and unless the mud is so stiff as to necessitate considerable rubbing they will be little subject to break down. This is borne out by the fact that in the case of softer muds, such as those from Stations 14 and 16a, the sums of Grades III and IV, into which the pellets would break down, show no steady rise with increasing depth. They do, however, show such a rise in St. 11 which is more clayey.

TABLE

Elutriation Grade

Expressed as percentage

Station.	Grade.	0–2·5.	2·5–5.	5–7·5.	7·5–10.	10–12·5.	12·5–15.	15–17·5	17·5–20.	20–22·5.	22·5–25.	25–27·5.	27·5–30.	30–32·5.
1.	1.	9·2	1·1	4·5	2·0	1·0	0·9							
	2.	67·2	72·5	26·9	69·8	64·8	65·2							
	3.	0·5	0·6	30·8	0·6	14·8	16·4							
	4.	23·1	25·7	37·8	27·6	19·4	17·2							
6a.	1.	11·7		2·4		12·1		2·7						
	2.	32·1		36·6		15·0		31·4						
	3.	27·9		0·7		43·5		35·2						
	4.	28·3		60·3		29·4		30·8						
8.	1.	8·9		0·9		5·9		2·5		2·2				
	2.	43·2		34·1		45·1		62·6		37·9				
	3.	5·7		28·1		1·0		25·9		0·1				
	4.	42·2		36·9		48·0		9·1		59·8				
8b.	1.	10·6				11·3		9·4		0·9				
	2.	33·0				38·6		19·9		16·2				
	3.	25·8				36·9		49·1		1·9				
	4.	30·6				13·2		21·6		81·0				
9.	1.	16·5		14·0		2·7		1·5						
	2.	33·7		23·3		33·8		29·9						
	3.	19·6		27·2		0·6		1·7						
	4.	30·2		35·5		62·9		66·9						
11.	1.	13·0	25·9	14·5	7·0		8·1	3·2	4·2	2·1	2·2	3·7	1·9	
	2.	41·5	33·1	41·5	46·5		29·1	24·4	13·4	18·4	27·4	24·2	15·9	
	3.	1·7	20·7	17·1	16·5		47·1	57·5	69·0	59·0	50·7	52·7	10·0	
	4.	43·8	20·3	26·9	30·0		15·7	14·9	13·4	20·5	19·7	19·4	73·2	
11b.	1.	9·0	4·9	1·9	13·0	2·4	3·0	3·4	13·3	1·2		2·3	5·0	24·3
	2.	44·6	44·7	42·5	30·1	22·7	37·1	33·2	26·6	25·8		32·1	39·8	23·5
	3.	12·5	18·6	23·8	24·2	44·2	36·2	11·6	30·7	42·1		2·1	3·0	1·8
	4.	33·9	31·8	31·8	32·7	30·7	23·7	52·0	29·4	30·9		63·5	52·2	50·4
12.	1.	3·6		3·7	3·8	2·0	2·0	0·9	0·7	2·7	6·9	4·6		
	2.	32·6		43·7	62·4	50·2	50·3	40·3	20·5	55·3	56·0	78·7		
	3.	0·7		0·6	1·8	1·0	1·5	2·0	65·2	1·4	0·7	0·7		
	4.	63·1		52·0	32·0	46·8	46·2	56·8	13·6	40·6	36·4	16·0		
13.	1.	12·2	2·9	3·7	3·8	3·9	1·3	2·1	3·6	1·7	1·5	1·7	7·4	8·9
	2.	52·1	57·8	53·7	68·2	59·7	54·1	49·5	60·2	46·6	47·7	50·1	48·3	38·2
	3.	3·4	3·2	3·4	2·2	15·2	2·0	21·8	3·8	26·5	20·6	3·4	1·4	1·8
	4.	32·3	36·1	39·2	25·8	21·2	42·6	26·6	32·4	25·2	30·2	44·8	42·9	51·1
13a.	1.	15·2	6·3	2·2	9·7	1·9	0·9	1·6	1·5	1·9				
	2.	71·3	57·7	72·4	56·8	41·3	38·7	33·9	39·9	40·0				
	3.	1·6	1·2	1·3	2·8	29·2	17·7	38·9	25·3	9·7				
	4.	11·9	34·8	24·1	30·7	27·6	42·7	25·6	33·3	48·4				
14.	1.	6·1	11·3		3·7	7·2	3·1	10·0	10·6	6·4	2·1	3·5	6·2	
	2.	52·3	47·8		56·0	53·4	64·4	53·2	56·8	43·6	53·3	50·5	51·9	
	3.	1·4	14·0		16·8	1·3	6·1	1·5	1·9	1·9	1·3	2·6	1·2	
	4.	40·3	26·9		23·5	38·1	26·4	35·3	30·7	48·1	43·3	43·4	40·7	
15.	1.	0·6	6·2	13·7	12·5	12·8		2·5	2·5	3·8	2·2	1·4	7·3	
	2.	65·7	58·6	83·0	55·3	47·7		52·8	44·2	46·3	29·3	20·4	16·0	
	3.	0·9	3·4	0·4	0·9	0·8		0·6	0·5	0·7	0·4	60·8	70·0	
	4.	32·8	31·8	2·9	31·3	38·7		44·1	52·8	49·2	68·1	17·4	6·7	
15a.	1.	29·8	21·9		2·3	2·4	6·4	3·6	3·7	5·6	8·3	5·7	11·7	
	2.	33·4	61·2		34·9	30·1	34·5	35·8	38·8	31·3	26·8	23·7	15·8	
	3.	1·2	1·2		1·0	30·3	9·5	2·3	1·0	28·0	29·6	28·0	38·9	
	4.	35·6	15·7		61·8	37·2	49·6	58·3	56·5	35·1	35·3	42·6	33·6	

VI.

Percentages.

dry weight.

Station.	Grade.	0–2·5.	2·5–5.	5–7·5.	7·5–10.	10–12·5.	12·5–15.	15–17·5.	17·5–20.	20–22·5.	22·5 25.	25–27·5.	27·5–30.	30–32·5.
16.	1.	3·6	4·9	4·2	5·6	5·3	6·8	4·7	2·4	2·1	2·4			
	2.	45·7	34·8	32·9	31·1	36·9	39·5	41·5	42·2	43·8	37·7			
	3.	1·3	0·3	2·3	1·9	37·4	30·8	27·1	20·3	5·2	14·2			
	4.	49·4	60·0	60·6	61·4	20·4	22·9	26·7	35·1	48·9	45·7			
16a.	1.	4·6	13·6	6·2	2·1	1·1		1·0	1·0	0·7	1·3	1·2	0·5	
	2.	45·6	44·2	37·9	47·5	46·6		48·7	45·6	43·8	50·6	42·0	46·1	
	3.	1·6	18·7	30·6	24·2	28·0		26·1	30·3	32·7	22·6	1·3	1·3	
	4.	48·2	23·5	25·3	26·2	24·3		24·2	23·1	22·8	25·5	55·5	52·1	
17.	1.	23·9	5·5	2·9	3·7	1·3	9·9	14·3	7·8	1·6	0·8			
	2.	1·7	3·7	48·7	35·1	43·8	28·8	0·8	26·5	15·8	19·5			
	3.	8·3	48·0	1·0	0·7	0·6	0·4	46·4	18·3	56·4	53·5			
	4.	66·1	41·9	47·4	60·5	54·3	60·9	38·5	47·4	26·2	26·2			
17a.	1.	83·1	84·4	83·9	76·2	80·6	86·9	89·5						
	2.	8·1	7·5	7·9	9·5	8·3	8·1	7·5						
	3.	6·5	4·5	0·1	5·5	0·1	0·2	0·2						
	4.	2·3	3·6	8·1	8·8	11·0	4·8	2·8						
17b.	1.	11·6	26·3	21·1	22·2	16·8	2·5	3·6	2·1	1·5	0·5	1·0	1·3	1·4
	2.	19·3	2·0	21·6	25·1	42·1	57·7	9·4	19·5	7·8	13·7	9·8	1·7	24·5
	3.	2·2	14·2	0·8	0·9	26·1	1·2	32·4	32·2	39·5	1·1	38·2	60·4	1·4
	4.	66·9	57·5	56·5	51·8	15·0	38·6	54·6	46·2	51·2	84·7	51·0	36·6	72·7
17c.	1.	22·9	18·9	20·1	14·1	13·0	14·4	2·3	0·9	1·8	22·3	15·3		
	2.	3·2	29·0	37·0	32·3	31·2	31·6	10·0	25·1	23·0	24·0	27·4		
	3.	14·4	0·6	0·5	1·0	0·7	1·1	61·7	0·9	17·3	16·9	2·3		
	4.	59·5	51·5	42·4	52·6	55·1	52·9	26·0	73·1	57·9	36·8	55·0		
18.	1.		19·4		8·5		7·8		14·5		10·7			
	2.		30·0		24·5		50·2		25·7		24·1			
	3.		30·4		35·0		5·1		46·6		48·6			
	4.		20·2		32·0		36·9		13·2		16·6			
19.	1.		3·6		5·3		10·2		1·7		1·1		3·8	
	2.		3·4		32·4		22·1		19·4		4·6		21·2	
	3.		37·9		39·3		51·2		44·3		61·5		48·2	
	4.		55·1		23·0		16·5		34·6		32·8		26·8	
20.	1.	86·3	34·4	18·9	1·2	12·2	9·1	15·5	18·2	41·1				
	2.	0·4	9·8	1·2	0·6	0·7	11·9	12·0	0·6	1·9				
	3.	10·2	23·1	46·2	56·9	47·7	34·3	48·9	65·4	32·8				
	4.	3·1	32·7	33·7	31·3	39·4	44·7	23·6	15·8	21·2				
23.	1.		5·0		2·1		1·6		3·1		2·2			
	2.		1·2		2·6		5·9		2·7		4·2			
	3.		23·3		41·8		61·5		75·4		71·8			
	4.		70·5		53·5		31·0		18·8		21·8			
24.	1	8·9	8·8	5·7	3·8	2·7	2·4	2·7	2·6	1·7	3·9	1·8	12·7	
	2.	37·4	22·1	26·8	10·9	13·7	7·0	11·8	0·2	3·8	6·3	1·9	1·7	
	3.	7·0	5·8	4·9	25·0	2·0	34·6	47·1	52·6	70·2	7·5	19·5	5·5	
	4.	46·7	63·3	62·6	60·3	81·6	56·0	38·4	44·6	24·3	82·3	76·8	80·1	
25.	1.		60·0				51·9		59·2					
	2.		27·9				29·5		27·8					
	3.		0·2				0·4		0·4					
	4.		11·9				18·2		12·6					
26.	1.	3·0	1·7	2·1	1·0	1·8	1·3	0·6	0·5	0·5	0·6			
	2.	51·4	42·4	47·4	48·6	41·6	41·4	53·9	47·4	45·5	56·2			
	3	1·9	1·4	33·3	37·5	1·0	1·1	26·0	3·0	0·7	1·7			
	4.	43·7	54·7	17·2	12·9	55·6	56·2	19·5	49·1	53·3	41·5			

Pellets such as those of Calanus which fall in large quantities on the mud surface, but are more friable than those of Maldanids, are broken down in the course of the elutriation.

Figure 5 shows the distribution of these four grades for St. 16a in the Holy Loch as an example of the mid-loch type; while the figures for all the stations are given in Table VI. The material falling on the mud surface is largely fine detritus with some larger particles of organic origin: this is reflected in the high value of Grade IV at the surface. Much of this

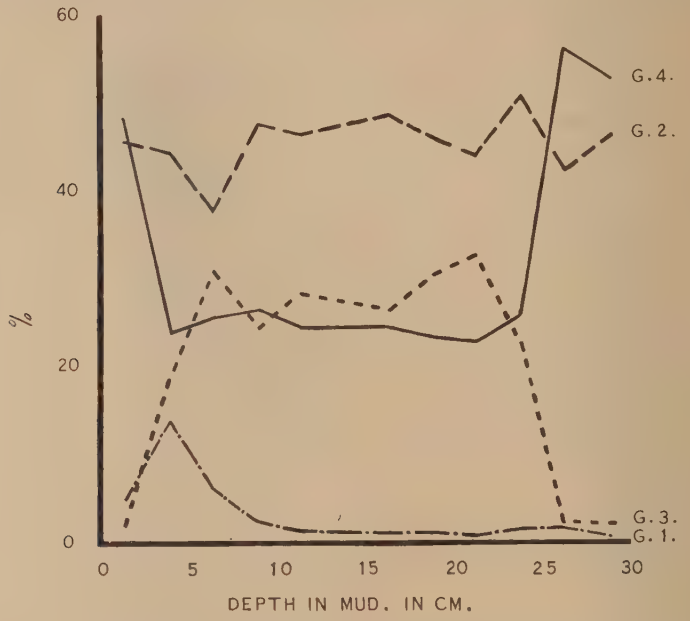

FIG. 5.—Distribution of particle grades with depth in the mud for St. 16a. G. 1 is the coarsest and G. 4 the finest.

fine detritus is eaten by animals living near the surface of the mud, and is converted by them into pellets. The effect of this is a rapid drop in the amount of Grade IV in the first 2 to 5 cm. and a corresponding increase in Grade I. Grade II is more erratic, and shows no clear general rise to correspond with this, though such frequently takes place a few centimetres deeper. Below this depth the mud is packed to a much harder consistency with the result that it needs more rubbing down, and Grade I decreases rapidly. This fall then is assumed to be largely artificial. At the same time natural break down of the organic particles causes a rise in the amount of Grade III in the first 5 to 10 cm. With the decrease in life

deep in the mud, there is little further pellet formation, and Grade II remains more or less constant. But the break down of the organic particles is still continuing, and at a depth of about 25 cm. Grade III suddenly drops to about 2% with a corresponding rise in the amount of Grade IV. The magnitude of this change may appear exaggerated since the difference in particle size in Grades III and IV is less than that between any other two grades. It points, however, to a steady slow break down of these organic particles.

As would be expected, at many stations conditions of deposition and change are not sufficiently regular to give as simple a curve as the one described. The type is best illustrated in the mid-loch stations, though the transitions in Grades III and IV usually take place more gradually than at St. 16a ; Stations 8b, 11, 16a, and 23 show the type fairly well, and Stations 9, 13, and 13a less clearly. The loch-head type of station shows much more erratic results, though St. 14 suggests this type. The stations in the Dunoon Basin are, as in other characters, very erratic, and the sandy stations are of course of quite a different nature. Station 20a shows a high proportion of Grade I in the two sandy layers, with a low Grade I and a high Grade III and IV in the intermediate mud layers.

There are then two distinct processes taking place in the mud. Firstly, the fine material which falls on the surface of the mud is being eaten and converted into larger aggregates in the form of pellets which form quite the most outstanding feature of the muds ; and secondly, there is a steady break down of the larger organic particles which fall on to the mud surface into finer material, many of these large particles being pellets, such as those of Calanus, but of a very much more friable nature than those formed in the mud. The pellet formation takes place only at the levels at which animal life exists, and chiefly close to the surface. Dr. Lloyd (**4**) has shown that bacteria are present in numbers in these muds in the deepest samples which have been taken, and perhaps due to them the break down of the organic particles continues to a greater depth.

Nature and Rate of Sedimentation.

With a view to determining the nature and origin of the sediments falling on the mud surface, and whether these showed variation in relation to any factors such as rainfall or plankton, collections have been made at various stations by means of two types of sediment pots.

The Type " A " pots are glass preserving jars, 32 cm. high, with an internal diameter of 9 cm. at the mouth. These were attached in string nets to a rope, buoyed at the surface. At the bottom of the rope was a lead weight, hanging just clear of the bottom at low water, and beyond this about 10 metres of slack rope attached to a heavy stone as an anchor.

The pots thus moved vertically with the tide, but hung more or less upright.

Another type of pot, " B," was used later in order to ascertain whether there was any lifting of bottom material once it had settled on the mud. The gear consisted of a series of seven smaller pots attached at 30 cm. intervals to a vertical iron rod, and this connected by a ring to a concrete block. A rope was attached to the top of the rod, and a set of submerged glass floats twenty metres above the rod kept the latter upright as well as keeping any slack rope clear of the pots ; the floats were connected

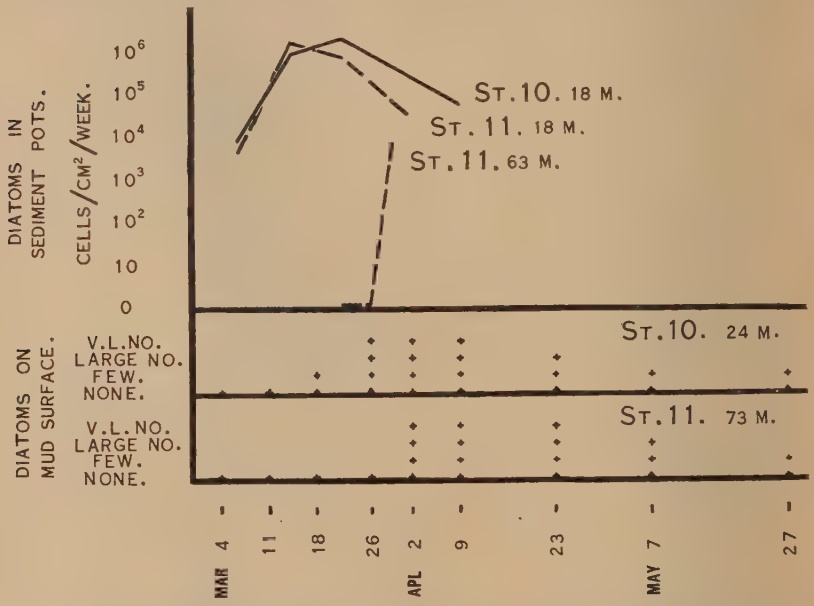

Fig. 6.—Occurrence of diatoms in the sediment pots (upper part of figure) and on the mud surface (lower part of figure) ; showing their appearance in sequence at the upper pots, the lower pots and the mud surface.

by a slack rope to a buoy at the surface. The pots used here were preserving jars, 17 cm. high, with an internal diameter at the mouth of 5·5 cm.

Type " A " pots were put out first at Stations 10 and 11. At St. 10, at Loch Striven Head, where the depth of water is 24 metres, only one pot was attached at a depth of 18 metres. At St. 11, at the middle of the same loch, one pot was attached at 18 metres, and one just clear of the bottom at 68 metres, the depth of water being 73 metres.

The gear was first put out on February 25th, 1930, and the pots were replaced at first weekly, and later fortnightly. At the same time vertical

hauls were made for plankton and tube samples of the bottom were taken for examination of the mud surface. Where possible diatom counts were made in the sediments by stirring up the latter with sea-water, and counting aliquot samples of it. The sediments were then washed, dried at 100° C. and weighed. Some difficulty was experienced with the bottom pot at St. 11 touching the bottom, so that records for this pot were not obtained until March 18th. Also diatom counts in this pot later became impossible owing to the large numbers of fæcal pellets of Calanus which were present. Both the diatom numbers and the sediment weights are expressed as the amount falling on one square centimetre in one week. Since *Skeletonema costatum* formed 95% of the diatom population at this time, other forms were neglected in the counts.

At St. 11 the diatoms reached a maximum at the surface of the water on March 25th and about three days later at the 10 and 20-metre levels. In the sediments from St. 10 and the top pot of St. 11 the diatom numbers run a very similar course (Fig. 6), reaching maxima of two million at St. 10 and one million at St. 11 in the weeks March 11th–18th and 18th–25th respectively, and then decreasing again. At the same time a count in the bottom pot at St. 11 showed no diatoms for the week March 18th–25th, but 3,800 the following week. Although no actual count of the numbers of diatoms on the mud surface was possible, it was clear that they appeared there quite suddenly and were present for a time in enormous quantities. At St. 10 they appeared first on the mud surface in small numbers on March 18th, and were present in very great numbers the following week. At St. 11, in much deeper water, they were not found on the mud until April 2nd, when, as at St. 10, they were present in great quantity. They continued very numerous at St. 10 up to April 9th ; on the 23rd they

TABLE VII.

NUMBERS OF SKELETONEMA CELLS IN SEDIMENT POTS DURING 1930.

Expressed as number of cells per sq. cm. per week.

Date.	St. 10.	St. 11. Top Pot.	St. 11. Bottom Pot.
March 4th–11th . .	13,700	11,500	—
,, 11th–18th . .	987,000	1,040,000	—
,, 18–26th . .	2,000,000	669,000	0
,, 26th–April 2nd .	—	84,000	3,800
April 2nd–9th . .	103,000	—	—

were definitely less, and on May 7th there were few left. In the same way, on St. 11, they were still numerous up to April 23rd ; on May 7th they were decreasing in numbers, and by the 27th there were few left, though,

of course, some are to be found on both stations all through the summer. The results are shown graphically in Figure 6 and the actual numbers in Table VII.

TABLE VIII.

Weights of Sediments in Sediment Pots During 1930.

Expressed as mg. per sq. cm. per week.

Date.	St. 10.	St. 11. Top Pot.	St. 11. Bottom Pot.
February 25th–March 4th	6·9	0·54	—
March 4th–11th . .	4·0	0·79	—
,, 11th–18th . .	9·4	0·71	—
,, 18th–26th . .	9·2	2·5	24·7
,, 26th–April 2nd .	4·2	3·4	33·4
April 2nd–9th . .	7·8	4·3	25·9
,, 9th–23rd . .	—	1·0	7·4
,, 23rd–May 7th . .	7·1	1·8	9·8
May 7th–27th . .	—	1·1	3·3
,, 27th–June 14th .	—	0·24	0·27

The weekly weights of sediments for the three pots are shown in Figure 7, and the actual quantities in Table VIII. Those for the top pot at St. 11 show a strong peak following the diatom increase in the water, but about three weeks later ; and the weight is greatly in excess of that which could be due alone to the diatoms in the pot. This excess is due to the presence of the pellets of *Calanus finmarchicus* of which the sediment was just then almost entirely composed. The vertical tow-nettings showed that these copepods began to appear in large numbers just after the spring diatom increase ; and at the same time their pellets first occurred in the pots. The suddenness of the drop in sediment weight after April 9th suggests that the Calanus shoals were no longer to any great extent feeding or at any rate shedding their pellets –at this level in this particular area. The weights of sediment for the bottom pot at this station follow a similar course, with a peak following the spring diatom increase maximum, though for no obvious reason the peak comes a week earlier in the case of the bottom pot. During the " Peak," the actual weight of the sediment is considerably greater at the bottom than in the upper pot, but later in the season they show little difference, i.e. 0·24 mg. in the upper pot, and 0·27 mg. in the lower. The pellets in the pots show that the difference is due, at any rate largely, to swarms of Calanus and Euphausids.

The constitution of the sediment is very similar in the two pots. In both there is a considerable amount of fine detritus of unrecognisable origin, although much of it clearly comes from the plankton ; a few

quartz grains up to 0·2 mm. in length, though usually smaller, may be present. The main bulk of the material in both pots during the summer is pellets, those of Calanus being predominant in the top pot and mixed with the larger pellets of Mysids and Euphausids in the lower pot. There are always a certain number of dead animals from the plankton, i.e.

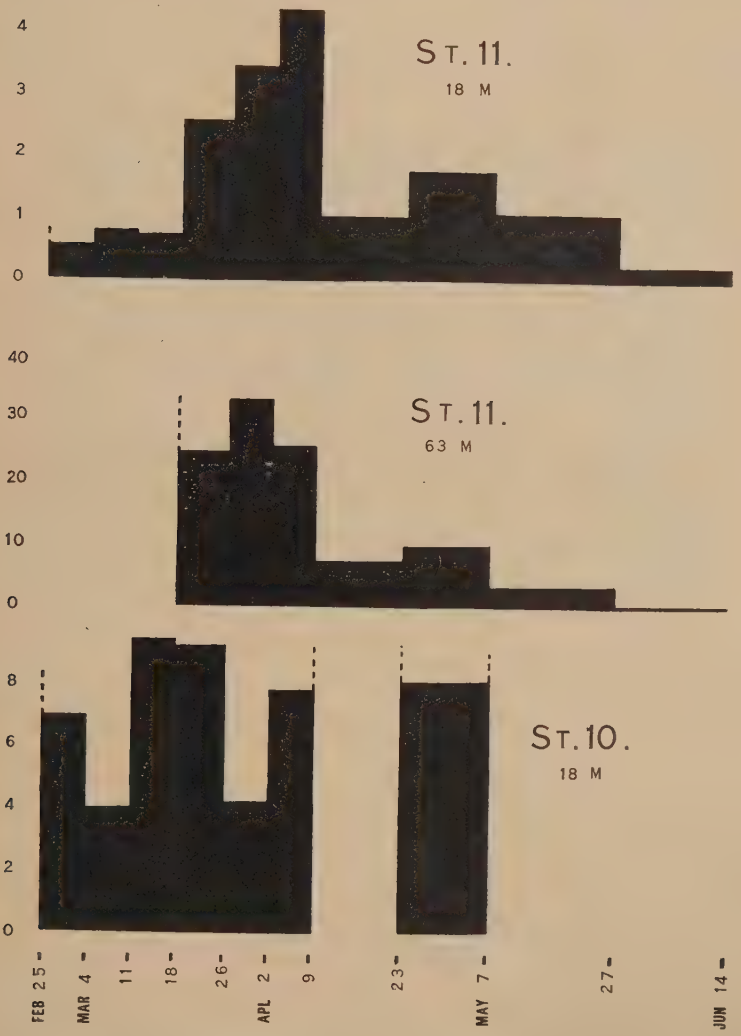

FIG. 7.—Weights of sediments—in mg. per sq. cm.—collected in Loch Striven during the period of the spring diatom increase. Note the vertical scale is different in each example.

copepods, rotifers, etc., as well as diatoms, the latter sometimes in very great quantity. At certain seasons, too, young lamellibranchs and polychætes are very common in the pots.

The sediment weights from the pot at St. 10 are very much more erratic than those from the other station, and show no " Peak " correlated with the spring diatom increase. In general the weight is considerably higher than that for the pot at the same depth at St. 11, and the sediment generally contains much more sand. Since we know that the contribution to it from the plankton, as reflected by the numbers of diatoms, is very

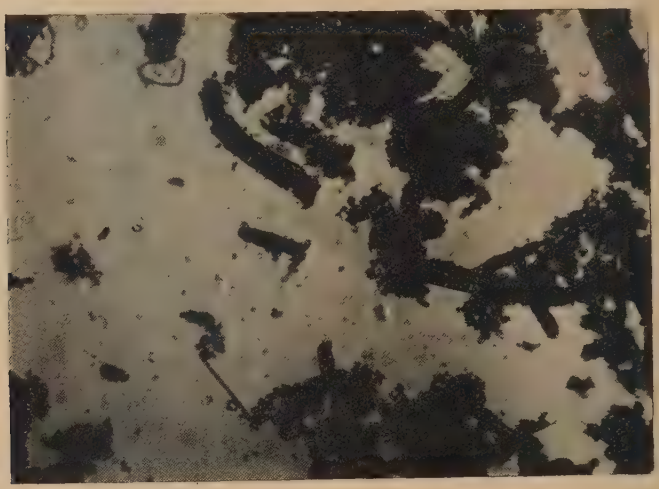

Fig. 8.—Sediment from St. 17d, showing Calanus fæces, diatoms and sand grains.

similar on both stations, the extra material must come either from the land or from the beach. If the former were the case we should expect to find some correlation between the amount of sediment and the rainfall and this has been found not to be the case. At the same time it does bear some relation to the strength of the northerly component of the wind, and it appears that this wind tends to lift sand off the banks at the sides of the loch in this region, and to carry it into deeper water. On St. 11 no such relation is found between rainfall or wind and the amount of sediment or the occurrence of sand grains in it.

In order to compare these results with those for less sheltered positions, two other stations were chosen, and pots put out at each of these for two periods of over a week. The first position chosen was St. 17d in Kilchattan Bay. The depth here is 22 metres, and the bottom sandy and

steeply shelving, and the bay itself fairly sheltered. One pot was placed as before at a depth of 18 metres. The weights of sediment per cm.^{2} per week were as follows :—

May 22nd to June 5th . . .	3·42 mg.
June 5th to June 14th . . .	5·37 mg.

The nature of the sediment was very similar to that obtained at this time in the lochs, i.e. mainly Calanus pellets together with some Euphausid pellets, and a few diatom chains. There was also a small amount of fine detritus and some sand grains up to 0·5 mm. in length, these being more abundant during the second period : one of these sediments is shown in Figure 8. The greater amount of sediment in this second period was due to the drifting of sand particles offshore, the wind being almost continuously from the east for the first period, and from the north-west for the second.

The pots were then put down at the same depth at St. 1c off Fairlie sands. The bottom here is sandy and exposed to a strong tide, and inshore there are extensive beds of Zostera. The first time there was a small whiting in the pot, so that the results may be too low. The material was almost entirely sand, together with some Calanus pellets. Some fragments of decayed Zostera were present, but these were very few considering the extent of the Zostera beds close to the pots, and as in the case of the Danish waters the weed is no doubt being carried in large masses into deeper and quieter water before it settles (Ostenfeld, **8**). The weight of sediment per cm.^{2} per week was 20·3 mg. The following week the Type " B " pots were used, and showed very similar results, with evidence that much of the material was being swept from the inshore sandbanks, there being pellets of Nucula in the pot 90 cm. above the bottom, and much sand in all pots. The average weight of sediment for the pots was 33·3 mg.

The Type " B " pots were also used on St. 11 in Loch Striven to ascertain whether there was any lifting of bottom material once it had been deposited. Their evidence is not conclusive, but it may be inferred from it, together with the presence of undisturbed diatoms on the extreme surface after the spring increase and also from the evidence of layering which is discussed later, that no such lifting takes place in these sheltered waters.

The results from these pots for a typical period are given below ; the top of No. 1 pot lying about 20 cm. above the mud surface, and that of No. 7 pot about 230 cm. above it.

Wt. of Sediment in mg. per cm.^{2} per week	1.	2.	3.	4.	5.	6.	7.
	0·72	0·66	0·65	0·59	0·54	0·52	0·44

In the bottom pot—No. 1—there were a few Maldanid pellets indicating slight disturbance of the bottom in lowering the gear, but this did not affect any of the higher pots. The material in the latter was partly fine detritus, but chiefly pellets ; these were mostly from Calanus in the top pot -No. 7 -but with a great many large Euphausid pellets in the lower pots. Although no count was possible, the number of these seemed sufficient to account for the increase in weight near the bottom, where the Euphausids are most abundant. If any lifting of bottom material had taken place, recognisable, small bottom pellets would have been expected at any rate in No. 2 pot and none were found.

It is interesting to compare these results with those given by Petersen for Thirsted Bredning in 1910 (Petersen, 9). These give an average of 6 mg. of sediment per sq. cm. per week ; and if we neglect the fourth, which was taken in much shallower water than the others, they give a striking suggestion of a spring maximum similar to that found in Loch Striven. Petersen states, however, that the sediment collected consisted almost entirely of fine detritus of unrecognisable origin, and that the plankton contributed little to it.

No.	Date.	Depth in m.	Sediment weight in mg. per sq. cm. per week.
1.	April 1st to April 15th	12	1·36
2.	April 15th to April 22nd	12	3·51
3.	April 25th to May 21st	14	15·68
4.	June 4th to July 5th	7	(0·49)
5.	July 6th to August 10th	10·5	8·96

Estimation of Rate of Deposition of Sediments.

If a tube sample of mud from a suitable locality is allowed to stand in a bright light or even in direct sunlight, it begins after a few weeks to develop alternate bands respectively lighter and darker than the original colour of the sample, and these may continue to intensify for from one to eighteen months. No such bands are visible in the fresh sample. While the light bands vary from 3 to 7 mm. in depth, the dark bands are narrow and not more than 1 or 2 mm. deep, and the pigmentation appears to be due to the formation of peat (see below). From a series of samples from St. 11, of which three, taken in February, early June, and July, are shown in Figure 9, it is clear that the dark band represents the sediment laid down at about the period of the spring diatom increase, and it is most probable that the colouration is due to the diatoms. At any rate it was shown in the previous section that there is a rich layer of diatoms on the surface of the mud on this station during the whole of April, and it is about this time that a new dark band appears.

No trace of diatom skeletons can be found in the layers, but they are probably an unstable form of silica which quickly disappears. The peat formation is probably associated with the very large numbers of bacteria

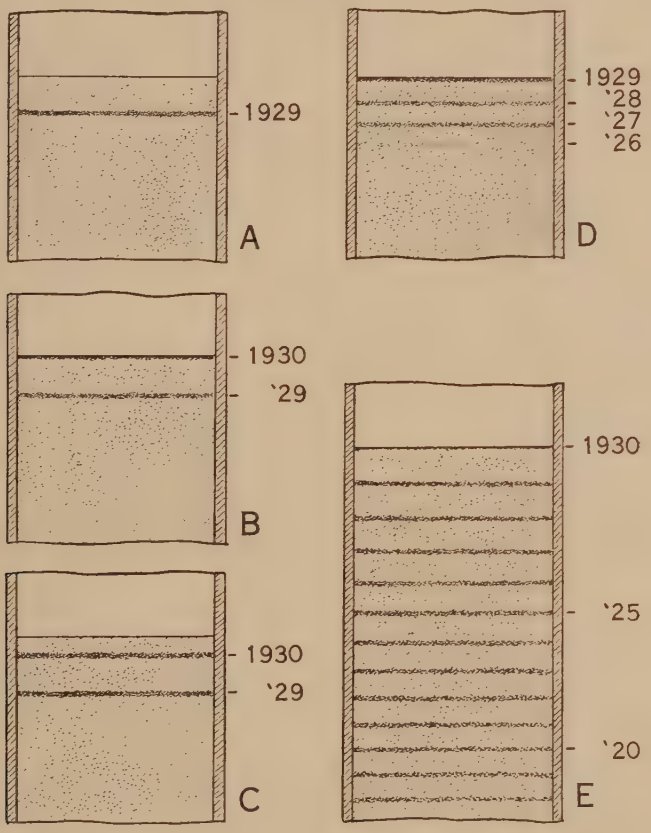

FIG. 9.—Annual layers as seen in tube samples of mud. The dark layers correspond to the spring diatom increase.

A. St. 11. 11th February, 1930.
B. St. 11. 5th June, 1930.
C. St. 11. 18th July, 1930.
D. St. 7. May, 1929.
E. Theoretical spacing of undisturbed bands for St. 11; packing practically ceases after ten years.

found by Lloyd (**4**) on the mud surface during the period of this rain of diatoms.

These bands are only found on stations where there is very fine sediment falling, and where there are no currents to disturb this once it has settled.

They are, therefore, not to be seen in the coarse mud of the Loch Head stations, nor those in the region of the Dunoon Basin where there are strong tidal currents, but they are well seen in the deeper ones at Stations 11, 7a, and 7b. On St. 11 the maximum number of bands which has been found is two, but on St. 7 four have been seen, the deepest rather faint. The non-appearance of bands below this is in all probability due to the mixing of the mud by the fauna, and this is in accord with the fact that on both stations the dominant forms, i.e. nematodes, are most abundant in the 1 to 2 cm. layer, while the layering is best seen in the top centimetre.

From the distance between the layers, it is possible to calculate the rate at which mud is being laid down at any station. For St. 11, the density and water content of the mud, being known, a graph can be obtained, giving the rate of packing of the mud with depth ; and from this, the theoretical spacing of the layers shown in Figure 8, E. At the surface the layers are 6 mm. thick, at about 5 cm. they have packed to 4 mm. thick, and beyond this depth there is little further packing. The top 5 cm. then corresponds to $9\frac{1}{2}$ years' sedimentation, and below this 40 cm. corresponds to about 100 years. At St. 7 the layers are 3·5 mm. apart at the surface so the rate of sedimentation is a little more than half that at St. 11.

The evidence that these are annual layers seems sufficient, but their spacing may be checked by comparison with the results from the sediment pots. If the water content at the surface of St. 11 is 85% and the density 2·3, the amount of material between the first two layers corresponds to 207 mg. per sq. cm. or a weekly average of about 4 mg. This is in agreement with the results from the bottom pot at the same place, which collected 100 mg. of sediment per sq. cm. during the 12 weeks which included the spring increase, or a weekly average of 8·3 mg. Considering the abnormally high values obtaining at the time, this is quite reasonable.

A similar type of stratification is found in the muds on the bottom of the Black Sea, although the conditions of deposition there are very different. The following is an extract (translated) from a paper by Schokalsky (**10**) in which he shows that the layers are probably the result of an alternation of a winter terrigenous deposit with a spring and summer planktonic deposit. Also, in the Black Sea muds, the sediment is undisturbed from the time that it is deposited, so that it is possible to trace layers layed down seven thousand years ago in the very long cores which he has taken.

" The fine stratification gives ten to twelve layers per millimetre ; it shows them best in samples taken near the coast. Each of the fine layers is composed of two, one black and organic in nature, the other grey and inorganic ; and we have likened each layer to an annual deposit. The black is the product of the plankton dying in winter, while the grey is the accumulation of debris carried down by rivers and coastal waters

in the spring. If we accept this hypothesis we arrive at the following results : a thickness of one centimetre is laid down in 50 years. In the layers of calcareous mud a similar stratification is observed, each layer again being double, one formed of calcium carbonate and the other of dead organic matter. From this we conclude that in any case the deposition of a column of mud of one metre will take about 4000 years."

I am indebted to Mr. A. Halimond for the following report on the geological nature of the layered Clyde Muds.

" Sections prepared by infiltration with artificial resin show a typical fine grained mud with small angular quartz grains and flakes of mica in a clayey ground mass. Small brown pellets are common, and there are also larger circular and elliptic areas containing in the centre the same minerals as the mud, but richer towards the margin in an isotropic brownish material, probably phosphate. A section through the top centimetre of the mud (E. 27845) shows a clear mud next to the surface, while at a depth of about half a centimetre there occurs a layer containing particles of brown amorphous ground mass which are probably either peaty or phosphatic matter.

At a depth of nine centimetres (E. 27844) the mud is generally similar to the lower layers of the surface."

The circular and elliptical bodies referred to above are, of course, the fæcal pellets of Maldanid worms.

Distribution of Fauna.

Although the sea bottom of the Clyde Sea Area has been very thoroughly worked from a faunistic point of view, such quantitative work as has been done has all been restricted to the shore and immediately below low-tide mark, and even here only horizontal and not vertical distribution in the substratum has been described in detail. In the deep water, the fauna may be divided into two main groups. The first comprises the large forms which either live at the surface of the mud, or can come up to it at intervals to breathe, and also the burrowing and tubicolous forms which, while penetrating to a considerable depth, maintain an open connexion with the overlying water. Of this type we have taken *Calocaris macandreæ* in its burrow at a depth of 20 cm., and tubes of Sabellid worms extending to a depth of over 30 cm. To take these in quantity, however, an instrument holding a large amount of mud would be necessary, and this has not been available.

The second group comprises those forms sufficiently small to be contained in a layer of mud not more than about a centimetre thick, and whose environment is determined by the nature of this layer. Since the nature of the mud may change very considerably within a vertical space of two

centimetres, it is necessary to examine only forms of less than about five millimetres long, if they are to be correlated with the nature of the mud in particular layers.

Several tube samples were taken from each station, and the mud cut up into one-centimetre layers as soon as possible. This was then passed successively through sieves of 10 and 120 meshes to the inch, and any polychætes retained in the former were neglected; small nematodes were also retained sometimes, entangled in masses of detritus, and these were removed and counted. The whole of the material left in the 120-mesh sieve was examined under a binocular microscope, and the various living animals picked out. The results of counts on a Loch Head, a Mid-Loch, and a Deep Water Station are given in Table IX, and the distribution of the two commonest forms, namely nematodes and copepods, is

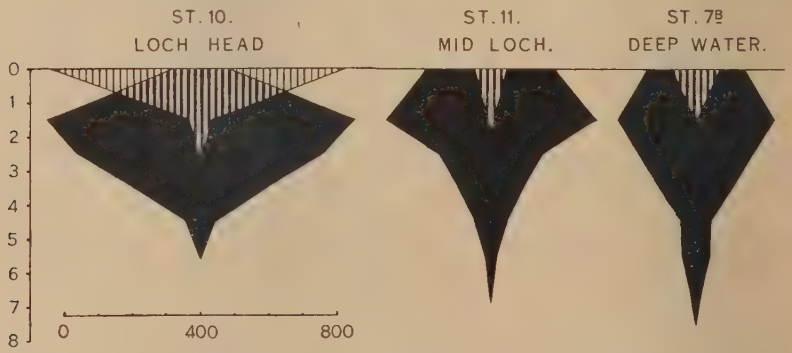

FIG. 10.—Distribution of the smaller fauna with depth in the mud on three types of stations: numbers of individuals—nematodes (black) and copepods (shaded)—in each one-cm. layer in a column of mud of 100 cm.^{2} surface area.

shown in Figure 10. Except in the surface layer, only very few ostracods and copepods were found, and these few individuals may either have straggled down from the upper layers, or else have been carried down into a deeper layer by the rim of the sampler. At any rate, they occur too rarely in the deeper layers to show definitely that they live there, and for this reason they have been neglected in Figure 10. The polychætes taken were mostly immature forms, and from their patchy distribution are not suitable for comparison.

All the copepods taken were Harpacticids, and they were limited almost entirely to the surface centimetre of the mud. Mr. Elmhirst examined a sample of these and found them to consist chiefly of *Danielssenia typica* Boek. and *Cletodes longicaudatas* Boek., with a few *Harpacticus uniremis* Kroyer. They are also very much more abundant in the Loch Head type of station than in either of those in deeper water,

the former showing a total density of 697 copepods per 100 sq. cm. while the other two show only 106 and 136. Ostracods show a very similar range, being also restricted chiefly to the top centimetre, and decreasing in abundance in the deeper stations, the total numbers per hundred square centimetres being respectively 125, 86, and 12.

The nematodes, on the other hand, show an entirely different type of distribution. Their maximum numbers in each station are attained in the 1–2-cm. layer and they are still found in quantity two centimetres deeper. While, as in the case of the copepods, the total densities decrease

TABLE IX.

Fauna Densities.

Numbers per 100 sq. cm. in each 1-cm. layer.

	Loch Head Type St. 10.				Mid-Loch Type St. 11.				Deep Water Type St. 7B.			
Depth in mud in cm.	*Nematodes.*	*Polychætes.*	*Ostracods.*	*Copepods.*	*Nematodes.*	*Polychætes.*	*Ostracods.*	*Copepods.*	*Nematodes.*	*Polychætes.*	*Ostracods.*	*Copepods.*
0–1	402	25	106	607	452	21	58	62	341	19	12	93
1–2	900	3	16	68	615	25	12	12	459	0	0	6
2–3	728	3	3	16	302	29	4	0	341	12	0	(19)
3–4	395	3	0	6	195	4	4	(4)	218	12	0	(6)
4–5	84	0	0	0	95	8	4	(12)	87	0	0	(12)
5–6	6	0	0	0	33	0	0	(8)	81	6	0	0
6–7	0	0	0	0	12	0	4	(8)	37	0	0	0
7–8	—	—	—	—	—	—	—	—	6	0	0	0
8–9	—	—	—	—	—	—	—	—	0	0	0	0
9–10	—	—	—		—	—	—	—	—	—	—	—
10–11	—	—	—	—	0	0	0	0	—	—	—	—
Total	2515	34	125	697	1704	87	86	106	1570	49	12	136

with increasing depth of water—namely, 2515, 1704, and 1570 respectively per hundred square centimetres—the depth in the mud to which they are taken increases in the deeper stations, the limits for the three stations being 6, 7, and 8 cm.

From the general similarity of the three types of station, together with the decrease in total densities of all forms in the deeper water stations, it would appear that either there is less available food in the deeper water or else other conditions are generally less favourable there. The chief differences between the three stations are the steadily decreasing rates of sedimentation with increasing depth of water, and hence a decrease in available food, and the much smaller oxygen avidity at the surface in St. 10. Now in all the muds, even at the surface, the oxygen content is zero, and for forms needing free oxygen this must necessarily be a limiting

factor. In view then of the experiments described below it is clear that the copepods cannot live for long in the absence of oxygen, and hence must live within easy reach of the oxygenated overlying water. The nematodes on the other hand are able to live in the absence of oxygen, at any rate for a longer period, so that the deeper layers, where they will have little competition, are available to them. Another factor, however, which must not be forgotten is the consistency of the mud, for it is clear that a copepod will find it difficult to move in a clayey mud, though able to do

FIG. 11.—Photograph showing mud converted into pellets by a Maldanid worm.

so easily in the loose surface mud, while a nematode from its shape and size is well adapted for movement even in the stiffer clays.

The reaction of the mud cannot be to any extent a limiting factor, since the differences found are so small, and also the differences between two stations in which the same species may occur are greater than those occurring within any one of the stations.

The larger forms are not taken in the sampler in sufficient numbers to allow any quantitative estimate of their importance. But they have a very great effect on the structure of the mud, since most of them feed on the finer particles either in the mud itself or falling from the water, and convert them in their guts into firm pellets. That this process is of extreme

importance in the muds is shown by the large numbers of pellets present. At St. 11, for example, 30% to 50% of the mud is converted into these pellets, and the average of a series of counts shows about 3400 pellets per cubic centimetre of mud. The effect of this aggregation on the effective particle size of the mud is obvious. In the first place, the liability of the mud to be carried by currents is greatly decreased, and in the second place, the mud is kept very much more open so that water can more readily diffuse into it. This will be clearly seen from an examination of Figure 11, although this certainly represents an abnormal sample in which practically the whole of the mud had been converted by a Maldanid worm into pellets. From a biological aspect also the process is important, since mud which has once passed through an alimentary canal and presumably lost much of its available nutrient material, instead of being set free to be re-eaten by some other animal is bound up in a distinctive form which will be refused by another selective feeder meeting it. This can be clearly seen in the case of a form like *Syndosmya alba* which lies buried, while its extended siphons feed indiscriminately from the surface of the mud. Periodically it blows out rejected material through one siphon, and this is found on examination to be largely composed of the pellets of other molluscs, worms, etc. The method of identification of these pellets, with special reference to those occurring on St. 11, is given in another paper (Moore, **7**). While accurate counts of the numbers of pellets are difficult, the following very approximate figures show the dominant form on St. 11.

Maldanids	3400 per c.c.
Nucula spp.	24 „
Syndosmya alba . . .	12 „

These results also demonstrate the value of pellet determinations compared with sampling of the actual animal by a " mud bucket " since the latter takes the two molluscs readily, but only occasional fragments of the deeper burrowing Maldanids.

Resistance to Anærobic Conditions.

In order to determine the ability of various of the mud-dwelling forms to exist in water containing no oxygen, the following tests were made. Animals were kept in the dark in stoppered bottles, in water from which as much oxygen as possible had been removed. This was done by alternately removing dissolved gas with a pump, and then saturating with washed hydrogen, and the oxygen content as determined by Winkler's method was reduced to less than 0·2 mg. per litre. In some cases this residue of oxygen also was removed by shaking with a small quantity of mud, but with no difference in results.

Syndosmya alba.

None lived for more than three and a half days, even in large vessels which were stirred twice a day with a stream of hydrogen. This is to be expected, since they normally keep a continuous connection with the overlying water by means of their siphons.

Nucula tenuis.

These lived and remained active for periods of from five to seventeen days, and death then may have been due to poisoning of the water by the animals' own excreta. The results are in agreement with the fact that Nucula is not provided with siphons like Syndosmya, and yet is found feeding at a depth of several inches below the surface of the mud.

Copepods.

Seventy-five per cent were dead at the end of 7 hours, and 90% at the end of 14 hours. A tube full of copepods in which all were apparently dead was opened at the end of 12 hours, and on exposure to the air, about two-thirds of them recovered, but after a longer period without oxygen there was no recovery.

Nematodes.

At the end of nine days 75% were dead and 90% at the end of a fortnight, while all the controls in oxygenated water were alive.

In these experiments the animals are undoubtedly in an environment very different from that of the mud, so that it is not legitimate to infer from them more than the fact that Syndosmya and copepods cannot live long in the absence of oxygen, but Nucula and the nematodes in question may be able to withstand it for comparatively long periods. In the case of the latter it would seem possible that they might live permanently without free oxygen. In an attempt more closely to reproduce natural conditions in the mud, the water in the tubes above a fresh sample of mud was replaced by deoxygenated water. The tube was then sealed at the bottom with paraffin wax, and the water covered with a layer of liquid paraffin. The tube was stored in the dark, and opened after thirty-five days. At the end of this time all the copepods were dead, but the nematodes were normal and active.

SUMMARY OF GENERAL CONDITIONS IN THE MUD.

The deep channels throughout the area have in general a bottom composed of mud. Where the bottom is sandy this is usually found to be associated with strong currents. The typical muds show a gradation from the type found at the heads of the lochs where streams enter, and the water is shallow, to those found in deeper water.

In all there is a steady deposition of fine material, the source of a large proportion of which being referable to the plankton in the overlying water. The chief differences in the material of the muds lie in the finer sediments in deeper water, and the coarser ones near the loch-heads, where large particles are contributed both by streams and by the beds of littoral and sublittoral algæ. The particles other than these vegetable remains reach the bottom in the form of fine detritus, diatoms, etc., and the fæcal pellets of forms like Calanus and various Euphausids ; and at different seasons the proportions of these vary, giving rise to very different types of sediment.

In these sheltered channels the surface of the mud appears to be sharply marked off from the overlying water, there being normally no mixing of the mud into the water. But the mud near the surface is in a very soft condition with a high water content of 80% to 85%. This soft condition may extend to a depth of 5 centimetres or more, representing about ten years' deposition, but by various agencies water is being pressed out of the mud, bringing it to a more clayey constitution, and at a depth of 20 centimetres it is usually stiff and comparatively hard. The mid-loch and deep-water types of station are alike in this, but the loch-head ones differ in that the mud is of a more open nature owing to the large organic particles present, and often also to an admixture of sand grains.

The nature of the mud changes considerably with increasing depth. The particles themselves are no longer the same. The friable pellets of the planktonic organisms are no longer recognisable and have probably broken down. At the same time much of the fine material has been aggregated by the various mud-dwelling forms into firm pellets which may last at any rate for a hundred years and probably for much longer. The chemical nature of the mud is also changing ; the total nitrogen and phosphate decreasing as well as the soluble phosphate, the latter decreasing most rapidly in the top five centimetres where most water is being expressed. Of special biological importance is the complete absence of oxygen even in the surface layers of the mud. The variation of pH is not great and has not been correlated with any other factors.

The relation of these factors has been studied chiefly with regard to the smaller organisms of which Copepods and Ostracods especially, and also Nematodes, are more abundant on the shallower stations. The distribution of all forms with depth in the mud seems to be regulated chiefly by their need for free oxygen and their mechanism for obtaining it. Syndosmya can burrow to the depth of its siphons, but dies quickly in the absence of oxygen. Nucula has no such siphons, but on the other hand is able to resist anærobic conditions of much longer periods, and can burrow to a considerable depth. Of the smaller forms with no such mechanism as that of Syndosmya, the various nematodes found are able,

like Nucula, to resist anærobic conditions for some time if not permanently, while the small crustacea, of which Harpacticids are the most abundant, have no such resistance, and are therefore confined to the extreme surface layers. Dr. Lloyd (**4**) has shown that bacterial life is abundant deep in the muds, but with the exception of tubicolous or burrowing forms nearly all animal life ceases at about ten centimetres.

Finally, I wish to express my thanks to the members of the staff at Millport who have assisted in the work.

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The Specific Identification of Fæcal Pellets.

By

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With 6 Figures in the Text.

In fine muds such as those covering most of the bottom in the deeper parts of the Clyde Sea Area the formation of fæcal pellets is a factor of great biological importance. In these muds up to 40 per cent of the fine material is often consolidated into fæcal masses, and in extreme cases even the whole of the mud may be in the form of pellets. The significance of this process is clear. In the first place, the effective particle size of a large proportion of the mud is very greatly increased, and this in turn affects such characters as the porosity of the bottom, and the liability of the material to be transported by currents. In the second place, among selective feeders such as *Syndosmya alba*, which feed on the mud, there is an economy of labour, in that food which has once passed through the gut is set aside in a form in which it will be refused if again taken in by the siphons. This latter process can readily be seen in the example cited whose rejecta on examination prove to be almost entirely composed of pellets of its own and other species.

The object of the present paper is to show the possibility of distinguishing between the various types of pellet occurring on any given ground. While it would be impossible to give a key distinguishing between all the types of pellets occurring in nature, it is probable that this may be done for any individual locality where the number of dominant species present is not too great. The locality chosen—St. 11—lies in a depth of 73 metres in Loch Striven, and is a very sheltered area with a soft mud bottom. The general nature of the mud here, as well as the fauna, is discussed in other papers (Moore, **4** and **5**).

Many mud-dwelling species, such as *Syndosmya alba* (Fig. 1), Nucula spp. and various polychætes, form pellets of very precise and unvarying shape. Others like *Syndosmya nitida* (Fig. 2) show much less regularity, while a few such as *Axinus flexuosus* void their fæces with no definite form. The majority of the important mud-dwelling species, however, form pellets, and these are surprisingly solid and resistant; they will stand fairly rough handling, and can even be boiled with sulphuric acid

FIG. 1.—Pellets of *Syndosmya alba*, ×60.

FIG. 2.—Pellets of *Syndosmya nitida*, ×36.

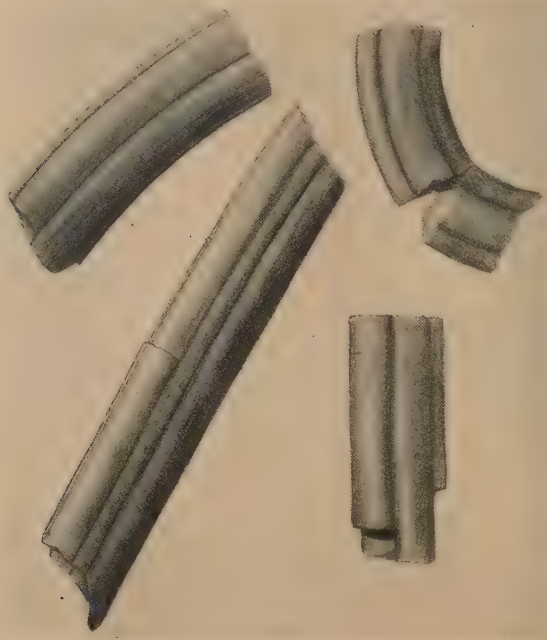

FIG. 3.—Pellets of *Nucula nucleus*.

or strong caustic soda without breaking down. On the other hand, the pellets of some of the planktonic forms such as Calanus, though quite definite in form, are much more easily destroyed, and are consequently not often identified except on the extreme surface of the mud.

The pellets have been examined both by collecting those shed by animals while under observation, and also by clearing the rectum in oil, or—as in the case of Nucula—by embedding the rectum in paraffin wax, and sectioning it. Except where stated, the following descriptions are the result of the examination of a number of specimens. There is a suggestion in the case of forms such as Calanus and Euphausids that the nature of the pellets may depend to some extent on the type of food which has been eaten. Dodgson (**1**) has found variation in the colour of the fæces of *Mytilus edulis* according to the nature of the food. The following are the chief types distinguishable on this station.

Nucula. The pellets of Nucula are voided in the form of a long rod of compact mud which breaks into lengths of 0·2 to 1·0 mm. or more, the breadth being about 0·15 mm. in pellets from an individual 1 cm. long. The intestine of Nucula shows thickened longitudinal ridges with long cilia, and these form grooves in the smooth surface of the pellet, whose number and position vary according to the species (Fig. 3). These pellets are sufficiently firm to allow identification in muds laid down on the bottom for over fifty years (Moore, **5**).

Nucula tenuis. Fig. 4, A.

Five very prominent equal longitudinal ridges; all the grooves are deeply cut, and the ventral pair are the most open.

Nucula nitida. Fig. 4, B.

Seven longitudinal ridges, the ventral one being smaller and sunk between the two on either side; none of the grooves are deeply cut, and in a rubbed or dirty pellet they may appear to be absent.

*Nucula nitida**—radiate form. Fig. 4, C.

Only five longitudinal ridges, but they are of the nitida type, with the lateral and ventro-lateral ridges fused to form one broad ridge; the grooves are shallow and the ventral ridge slightly sunk as in *N. nitida.*

Nucula nucleus and N. radiata. Fig. 4, D and F; and Fig. 3.

Seven longitudinal ridges as in *N. nitida,* but more prominent and with the grooves more deeply cut—sometimes more deeply than shown in the figure and even so much so as to allow the ridges to be split off separately.

* This form is distinct from *N. radiata,* but also according to its pellets distinct from *N. nitida.* I am indebted to Mr. R. Winkworth for its identification.

Fig. 4, F, shows diagrammatically the position of the grooves as seen in surface view, but identification of all these forms is easier by examination of the ends.

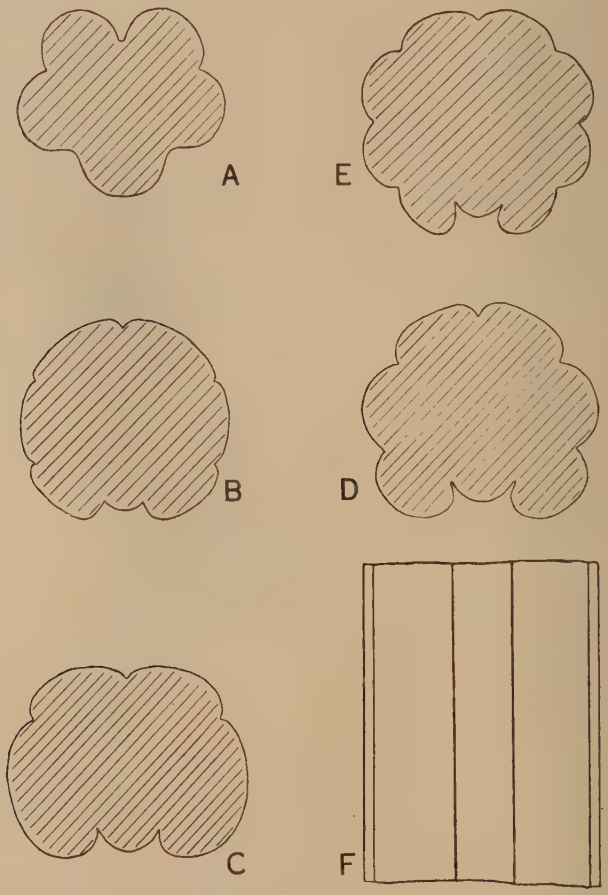

Fig. 4.—All ×200. A, Pellet of *Nucula tenuis*, end view: B, Pellet of *Nucula nitida*, end view: C, Pellet of *Nucula nitida*, radiate form, end view: D, Pellet of *Nucula nucleus*, end view: E, Pellet of *Nucula sulcata*, end view: F, Pellet of *Nucula nucleus*, plan of lower surface.

*Nucula sulcata.** Fig. 4, E.

Nine longitudinal ridges, separated by grooves of medium depth; the ventral ridge deeply sunk as in *Nucula nucleus*.

* Although occurring on this ground, no fresh specimens were available, and the above description and figure are from a single specimen supplied by Mr. A. C. Stephen from the east coast of Scotland.

Pecten septemradiatus. Fig. 5, A.

This also voids its pellets in the form of a long rod, but the surface is less smooth than in Nucula, and frequently marked by transverse clefts so that the pellet breaks into short lengths. In section the pellet is triangular with rounded corners, one being usually sharper than the other two, and with slightly concave sides. From a specimen 3·5 cm. across the shell the pellets are about 0·6 mm. broad and in lengths of one to three millimetres or more.

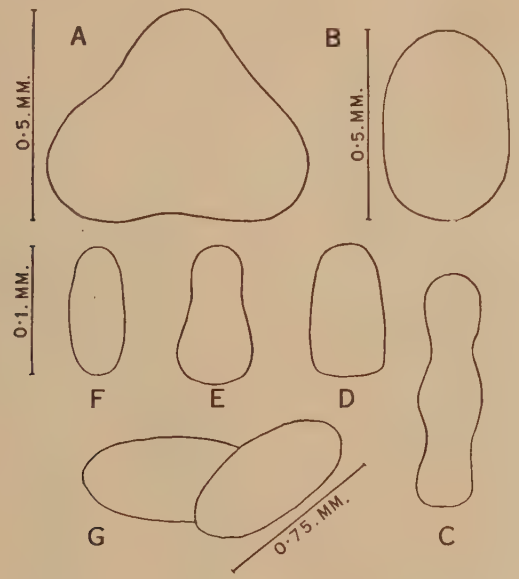

FIG. 5.—A, Pellet of *Pecten septemradiatus*, end view: B, Pellet of *Syndosmya alba*, seen from above: C, D, and E, Pellets of *Syndosmya nitida*, to same scale as B: F, Pellet of young Maldanid worm: G, Pellets of Maldanid–Rhodine sp.

Syndosmya alba. Fig. 5, B; and Fig. 1.

The pellets are extremely uniform in shape, parallel-sided with rounded ends and circular in section; the surface is smooth and regular; the usual breadth is 0·25 to 0·3 mm., and the ratio of length to breadth about 1·75.

Syndosmya nitida. Fig. 5, C, D, and E; and Fig. 2.

Much less regular in form than *S. alba*, and frequently either tapered at one end or constricted at one or more points; surface smooth; narrower than *S. alba*, breadth 0·2 to 0·25 mm.

Maldanids, various. Fig. 5, F and G.

There is a type of pellet very common in the mud, and not referable to any one species ; this is an elongated, oval pellet quite distinct in shape from that of *S. alba*, and of from 0·1 mm. to 2·0 mm. in length. Two examples are shown in Fig. 5, F, being that of a young unidentified Maldanid

Fig. 6.—A, Pellets of *Calanus finmarchicus* : B, Pellets of *Meganyctiphanes norvegica*.

about 4 mm. long common in the mud, and Fig. 5, G, a pellet 0·85 mm. long from the gut of a specimen of *Rhodine* sp. about 10 cm. long.

Of the planktonic forms whose pellets may be found on the mud, Calanus is the most abundant ; Sagitta, though very abundant, appears not to form a solid pellet. Euphausids, and to a lesser extent Mysids and Crangonids, are common near the bottom, but it is only possible to describe a single type of pellet common to all three.

Calanus finmarchicus. Fig. 6, A.

This has a rather transparent pellet usually 0·05 to 0·1 mm. in breadth and 0·2 to 0·4 mm. long. They vary considerably in shape, but are always elongate. The ends may be broken off, but one or both is often rounded or pointed, and one often drawn out into a long clear " tail " (Fig. 6, A). The constitution of the pellets has been described by Marshall (**3**) who shows that it is largely composed of diatoms and other plankton organisms, including a certain amount of crustacean fragments. The pellet is usually most transparent towards either end.

Euphausids. Fig. 6, B—i.e. *Meganyctiphanes norvegica*, *Nyctiphanes couchii*, and *Thysanoessa raschii.*

These have pellets somewhat similar to those of Calanus, but larger and more opaque ; the ends are sometimes rounded, but they are generally broken off and frayed ; crustacean remains are frequent (Macdonald, **2**), and large crustacean spines often project from either end of the pellet. They vary greatly in size, those of a 30 mm. Meganyctiphanes being about 0·1 to 0·15 mm. broad and up to 4 mm. long, though pellets collected on the bottom have always been found to be shorter than those shed in captivity. The above description applies also to the pellets of those Mysids and Crangonids occurring on this ground.

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Rays and Skates of Devon and Cornwall. Methods of Rapid Identification on the Fishmarket.

By

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With 10 Figures in the Text.

In carrying out detailed investigations into the life-histories and distribution of the Rays and Skates around the extreme south-west of England, large numbers of commercial landings have had to be examined. On account of their size and cost, regular and adequate samples of Rays and Skates cannot be delivered at the laboratory, as is possible with such a small and inexpensive fish as the Herring, for example. It therefore has been essential to learn rapidly to distinguish the different species as they lie exposed for sale on the fishmarkets. This has been necessary not only to promote rapidity of work, but also to obviate all unnecessary handling of the fish, too much interference with which is apt greatly to annoy fishermen, salesmen, and buyers alike.

As Rays and Skates are laid out for sale in lots often of one hundred or more all with their lower surfaces upwards, the task of identifying the species without raising each fish to examine its upper side was found at first to be one of extreme difficulty, and accurate determinations were not possible. But gradually, as the result of concentration and much practice, it became possible to identify—simply from the characters discernible on their under surfaces—the eleven species of Raia landed at the various ports of Devon and Cornwall. Only when this facility has been acquired is it possible to make a general survey of the stocks present on the various fishing grounds by dealing rapidly, amidst the bustle and confusion of a busy market, with large numbers of fish in commercial catches.

In this paper, therefore, an attempt is made to enumerate and describe as clearly as possible the distinguishing features which have been found most useful for rapid identification of the species landed in this area. The descriptions are applicable to fishes of marketable size, and must not be expected to apply to stages less than about 30 cm. across the disc

which do not ordinarily find a place in commercial landings. It will readily be understood, too, that minute details of obscure features, no matter how diagnostic they may be, will receive no mention.*

By careful observation of the characters here described any worker, even without previous experience of the family, should be able to distinguish with certainty and ease at least seven of the eleven species of Raia likely to be seen on the fishmarkets of Devon and Cornwall. The four others are less easily recognised, but a little patient practice should render possible the identification of them also from the characters normally visible on the under side. Nevertheless, even the experienced worker will encounter, from time to time, a fish which will require at least a glance at the upper surface to establish its identity. Occasionally, too, a specimen may occur which will be at once recognisable as either *Raia brachyura* or *R. montagui*—two short-nosed Rays often very similar in appearance—but cannot readily be assigned to its correct species even on careful examination of both sides. In such circumstances the easiest and most certain method of deciding the point, if several other undoubted examples of both species be available, is as follows :—

Lay out, back upwards, two separate lots of fish, one lot consisting of *R. brachyura* alone and the other of *R. montagui* alone. This done, place the doubtful specimen in the middle first of the one lot and then of the other ; it will then at once be obvious to which lot—and therefore to which species—it rightly belongs.

As indicated above, eleven species of Raia are landed more or less regularly at the various fishing ports of Devon and Cornwall. These are *R. montagui*, *R. brachyura*, *R. undulata*, *R. microcellata*, *R. clavata*, *R. naevus*, *R. circularis*, *R. fullonica*, *R. marginata*, *R. batis*, and *R. oxyrhynchus*.† These eleven species fall naturally into two main divisions : —

I. those whose under sides are of a decidedly dark ground-colour richly studded with black spots and which, for our present purpose, may be called " Black-bellied Species."

II. " White-bellied Species."

* For such detailed descriptions reference should be made to Clark's admirable monograph on the European species of Raia (1).

† Nomenclature according to Clark's monograph (1).

I. Black-bellied Species (Skates).

Only two species—*R. batis* Linn., and *R. oxyrhynchus* Linn.—are included in this division. They are separately distinguished as follows :

RAIA OXYRHYNCHUS.

Long-nosed Skate.
Bottle-nosed Skate.

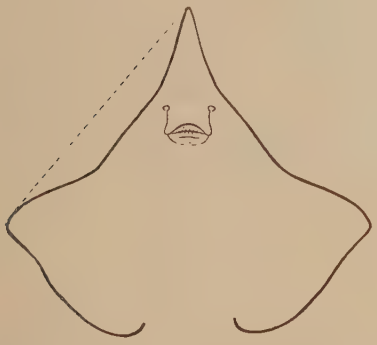

Fig. 1A.—*Raia oxyrhynchus*—outline of disc.

(Fig. 1A.)

Snout very long, narrow, and pointed.

Outline of anterior margin of disc strongly concave.

Rarely reaches a width of 100 cm. across the disc.

RAIA BATIS.

Common Skate.
Blue Skate.

Fig. 1B.—*Raia batis*—outline of disc.

(Fig. 1B.)

Snout relatively much shorter, broader, and less pointed than in *R. oxyrhynchus*.

Outline of anterior margin of disc only slightly concave, often nearly straight.

Grows to a very large size, specimens of 150 cm. in width being not uncommon.

Large Skates are generally picked out by the fishermen and spread separately on the fishmarket. The smaller sizes, up to about 50 cm. across the disc, are laid out with the Rays interspersed indiscriminately among them.

II. White-bellied Species (Rays).

These fall into three distinct groups, according to the shape of the disc.

1. LONG-NOSED RAYS . .	*R. fullonica* Linnæus. *R. marginata* Lacépède.
2. CIRCULAR RAYS . .	*R. undulata* Lacépède. *R. naevus* Müller and Henle. *R. circularis* Couch.
3. SHORT-NOSED RAYS . .	*R. clavata* Linnæus. *R. brachyura* Lafont. *R. montagui* Fowler. *R. microcellata* Montagu.

1. LONG-NOSED RAYS.

The two species included in this group both have long and pointed snouts which mark them out at once from all the others.

RAIA FULLONICA.

Shagreen Ray.

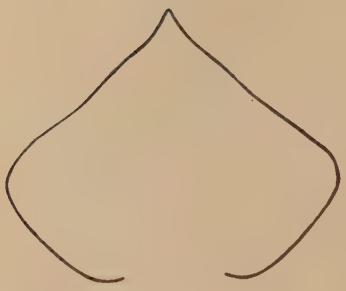

Fig. 2A.—*Raia fullonica*—outline of disc.

(Figs. 2A and 3.)

There is a well-marked groove (Fig. 3, G) in the middle of the upper jaw and a corresponding

RAIA MARGINATA.

Bordered Ray (Young).
White-bellied Skate* (Adult).

Fig. 2B.—*Raia marginata*—outline of disc.

(Figs. 2B and 4.)

Curve of mouth cleft quite regular.

* The large adults of this species are known to fishermen as "White-bellied Skates" and are laid out apart from the Rays, either by themselves or along with the large Blue and Bottle-nosed Skates. They are then very conspicuous because of the clear whiteness of their under sides, sharp snouts, and strongly undulated anterior disc margins.

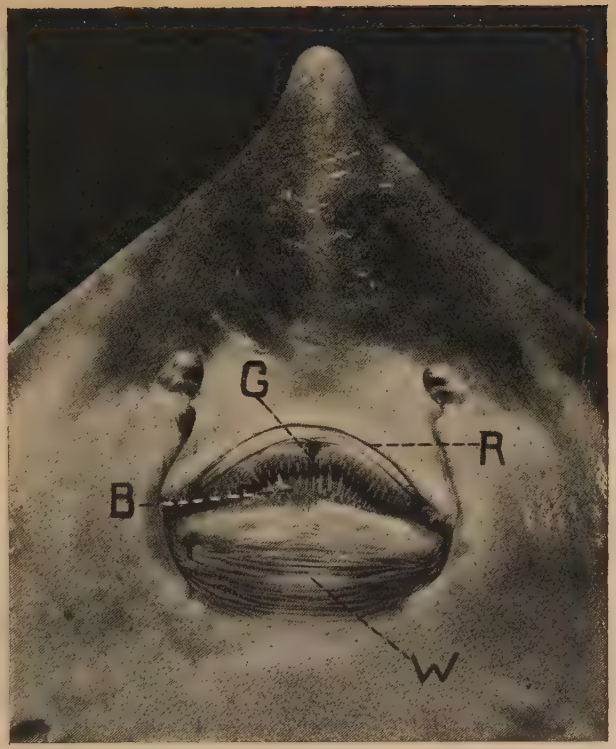

FIG. 3.—*Raia fullonica*—mouth and nasal regions.

R—pre-oral recess.
G—groove in middle of upper jaw.
B—" boss " in middle of lower jaw,
W—post-oral wrinkled region,

FIG. 4.—*Raia marginata*—mouth and nasal regions,

"boss" (Fig. 3, B) in the middle of the lower jaw which give the mouth cleft a very irregular outline.	
Internasal distance appreciably less than width of mouth.	Internasal distance about equal to, or slightly greater than, width of mouth.
Anterior margin of disc not strongly undulated.	Anterior margin of disc strongly undulated.
Ventral surface of tail white.	Ventral surface of tail dark-coloured, sometimes nearly black.*
Seldom grows beyond 70 cm. in width of disc.	Adults very large, up to 150 cm. across the disc.

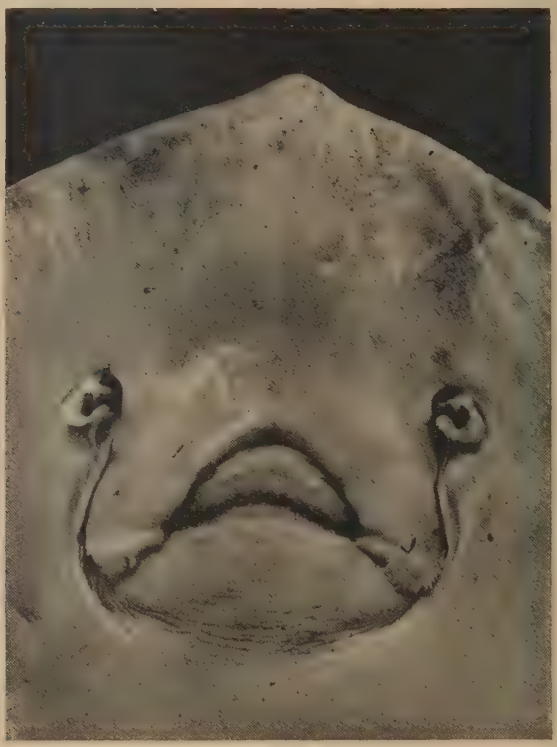

FIG. 5.—*Raia undulata*—mouth and nasal regions.

* In very young individuals there is a broad black band around the tips and along the posterior margins of the wings, but as this gradually disappears with age, it cannot be used with confidence for distinguishing the species.

2. CIRCULAR RAYS.

The three species which fall into this group all have the tips of their wings decidedly rounded and their snouts scarcely project in front of the main contour of the disc—characters which give them a typically "circular" appearance.

RAIA UNDULATA.

Marbled Ray.

(Fig. 5).

Arch of pre-oral recess high and acute.

Teeth flattened and close set, so that the separate rows are not discernible.

"Groove and boss" absent.

RAIA NAEVUS.

Cuckoo Ray.

FIG. 6A.—*Raia naevus*—outline of disc.

(Fig. 6a.)

Arch of pre-oral recess low and obtuse.

Teeth long, pointed, and in widely separated rows which show up clearly.

"Groove and boss" generally discernible.

Anterior margin of disc strongly undulated.

Fish very thick and fleshy.

The smallest of the Devon and Cornwall Rays, seldom exceeding 40 cm. across the disc.

RAIA CIRCULARIS.

Sand Ray.

FIG. 6B.—*Raia circularis*—outline of disc.

(Fig. 6b.)

Arch of pre-oral recess low and obtuse.

Teeth long, pointed, and in widely separated rows which show up clearly.

"Groove and boss" generally discernible.

Anterior margin of disc not strongly undulated.

Fish always appreciably thinner than a specimen of *R. naevus* of the same width across the disc.

Adults commonly as much as 70–75 cm. across the disc.

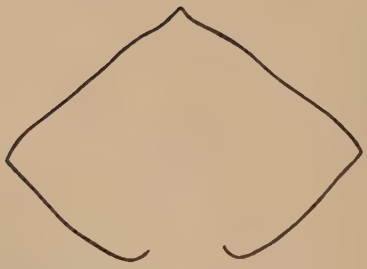

Fig. 7.—*Raia clavata*—outline of disc.

Fig. 8.—*Raia clavata*—mouth and nasal regions.

3. SHORT-NOSED RAYS.

In each of the four species included in this group, the general outline of the disc is typically diamond-shaped, due to the sharpness of the wing-tips and the presence of a short but well-defined snout (see Fig. 7).

RAIA CLAVATA.

Thornback Ray.

(Figs. 7 and 8.)

Arch of pre-oral recess high and acute.

Teeth large and distinct, markedly tessellated in adult females and in immature specimens of both sexes ; more pointed in adult males.

Internasal width about equal to width of mouth.

Lower surface with large spines and/or rough patches.

RAIA BRACHYURA.

Blonde Ray.

(Fig. 9.)

Arch of pre-oral recess low and obtuse (except in adult males).

Internasal distance always less than width of mouth.

Large spines never present on lower surface.

Outline described by oro-nasal canals and hinder margin of post-oral wrinkled area (see Fig. 3, W) forming a wide with nearly straight or even concave base.

Surface smooth all over except in large adults which may show some rough patches.

Often grows to 80 cm. in width of disc.

RAIA MONTAGUI.

Spotted Ray.
Homelyn Ray.

(Fig. 10.)

Outline described by oro-nasal canals and hinder margin of post-oral wrinkled area forming a more rounded with convex base.

Surface smooth at all ages.

Seldom grows beyond 50 cm. across the disc.

Fig. 9.– *Raia brachyura*– mouth and nasal regions.

Fig. 10.—*Raia montagui*– mouth and nasal regions.

RAIA MICROCELLATA.

Painted Ray.

Resembles *R. montagui* in the characters of the mouth and nasal regions, but the pre-nasal area is covered with small spinulæ. In large specimens these are easily seen, but in smaller individuals it sometimes is necessary to touch the surface with the fingers in order definitely to establish their presence or absence.

Adults often grow to 70 cm. across the disc.

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Tube Formation in *Pomatoceros triqueter* L.

By

Dr. H. Faouzi.

With 3 Figures in the Text.

As a serpulid, *Pomatoceros triqueter* L. realises to the highest degree the adaptation to a sedentary life. The calcareous tube in which this annelid lives is characterised by a keel running along its upper surface. It is rather misleading to say that this tube is triangular in section. Figure 2 shows a newly formed calcareous rod ; it is spur-shaped ; the two rami of the spur might approach each other so nearly as to simulate an almost complete tube. The substratum in this species not only serves as a support, but completes the tube. This is in accordance with the fact that the tubes of Pomatoceros never extend freely off the support as happens with tubes of other serpulids—Protula, for instance. The aim of this paper is to record some observations on the way an adult Pomatoceros endeavours to build a new calcareous tube if it is artificially removed from its old one. I undertook this observation as original work at Roscoff, where a complete literature was not available. I discovered later that Harms, in the course of his research on regeneration in *Hydroides pectinata*, arrived at identical results. My paper cannot therefore pretend to more than a confirmation of previous results.

The Pomatoceros is removed from its tube, and placed carefully in a flat-bottomed dish full of sea-water. The worm lies on its side, its abdomen curved in a dorsal direction, performing a forward and backward undulation. If the individual happens to be sexually mature, its first preoccupation is to throw off its sexual elements—pink eggs or whitish spermatozoa—in such a quantity that one can notice with the naked eye floculent material streaming out from among the gills, and colouring the water. Through the binocular it can easily be seen that these elements stream through the fæcal groove, from its posterior end, follow its bend at the thorax, until they leave the head on its dorsal surface, to pass freely into the sea-water. Once the annelid has exhausted its sexually-mature contents, nothing more notable is to be observed for some hours (usually 6 to 10). Then a certain turbidity begins to show in the fold of the collar, just in the middle third of each side of this organ. Figure 1 shows how in

the natural disposition of the collar there is a crest in the middle which divides it, so to speak, into two parts. This crest is orange-coloured normally and by the time the fold shows the white turbidity, this crest begins to whiten. A little later the turbidity definitely forms two white rods, occupying always the middle-third of both sides of the fold ; the crest follows by forming in its fold another rod. These white rods were proved to be calcareous, and the process itself showed subsequently how we were in presence of a newly forming tube.

Active secretion of the collar adds to the substance of the laterally-placed calcareous rods, and they lengthen in this way until they attain a position vertically above the calcareous crest already mentioned, in

Ventral view. Lateral view.

Fig. 1.—Microphotograph of the anterior half of a Pomatoceros deprived of its tube, to show the folded collar with a crest in the middle.

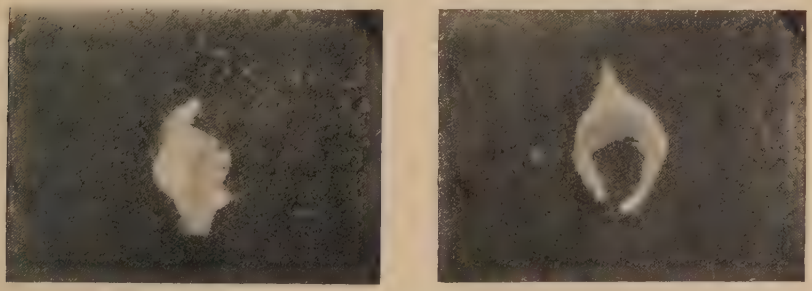
which they fuse, and we have at last a spur-shaped piece reproducing exactly the space in the fold of the collar, with its ventrally situated crest. Figure 2 shows a piece of this type formed by one of the worms and thrown accidentally off the worm. As a matter of fact, the least shock at this moment is sufficient to provoke this accident.

The next step is the lengthening of the spur-rami dorsally ; the free ventral extremities of the collar come nearer to each other but never to such an extent as to join the rami together. Here lies the explanation of the incomplete tube of the Pomatoceros of which mention was made above. This does not mean that where a complete tube is found in the serpulid a complete collar is to be expected. I have examined a Protula a species with a complete tube which has none the less an incomplete collar. The collar is so flabby in Protula, compared with that of Pomatoceros, so easily pliable as to be able to surround completely

the dorsal side of the thorax, a fact which facilitates the joining of both rami in this species and the formation of a complete tube.

Calcareous secretion goes on as described, but this time increasing the surface of the rod ; one should imagine a long series of spur-shaped pieces formed continuously and as a whole until a definitely-formed Pomatoceros tube is produced. The keel is due to the calcareous crest formed in the ventrally situated collar-fold. To come back again to Protula—a species with a completely round tube one notes the absence of this particular fold characteristic of serpulids with keeled tubes.

Pomatoceros is then capable of reconstituting its tube. This notion is rather theoretical, and practically these worms are unable to resist the all-powerful circumstances that surround them, once they lose their shell. I have succeeded in keeping alive a single Pomatoceros for forty



Lateral view. Face view.

FIG. 2.—Microphotograph of a spur-shaped calcareous piece, formed in the fold of the collar and removed accidentally. The point of the spur is the origin of the keel to be seen on the upper surface of tubes of Pomatoceros.

days ; I had to protect it and, so to speak, help it to live. One of the reasons for this difficulty must first be excluded, as it is the condition *sine qua non* of any calcareous formation whatsoever : the calcareous reserve once exhausted, the animal is unable to form a new tube. It thus happened at Roscoff that I found worms that either did not form calcareous rods at all, or, once these rods formed, secretion did not go on any further. These cases were rather rare at Roscoff between August and September. At Plymouth I tried to repeat the experiment with a view to procuring material for cytological examination ; on twenty worms removed by the method I described, not a single one gave evidence of regenerative activities. This happened in April, and points perhaps to some relation with sexual activity which did not manifest itself in April at Plymouth, while sexually mature Pomatoceros were the rule at Roscoff in August and September.

Then comes the absolute absence of defence on the part of the worm

deprived of its tube. Active planktonic fauna, floating in the aquarium tank, crustaceans and small annelids, surround the naked worm, in search of the least breach in the surface of its body to start their work of destruction.

As regards the Pomatoceros that I succeeded in keeping for forty days

Ventral view.

Ventro-lateral.

Left-lateral.

Right-lateral.

FIG. 3.—Pomatoceros which continued forming a tube for forty days ; the various views of the worm show the irregular tube, formed as usual but displaced from time to time owing to the continued movement of the naked serpulid ; the spur points do not correspond as they should do under normal conditions to form a single continuous keel.

(Fig. 3) I had to take many precautions : cleaning vessels daily, managing to get rather clean sea-water by passing it through a series of siphons, taking off particles of excreta and foreign matter from the body of the worm. The Pomatoceros went on forming a tube, but did not succeed in

fixing that tube. This is due to the continual movement of the abdomen. The ventro-dorsal movement proves very deleterious to the fine calcareous rods, lying loose in the fold of the collar ; the least shock is sufficient to displace these rods, or even the well-formed spur. It was by mere chance that one Pomatoceros out of many was able to go on secreting a continuous piece for forty days, and even in this case the calcareous ring was displaced, not enough to leave the worm entirely, but to turn in a circular direction, the keel coming to a lateral position. A study of the accompanying figures shows how irregular this tube is ; it presents as many keels as there were displacements : after each turning of the calcareous ring, the worm proceeded with its new tube formation, and thus the new keel did not come in line with the old one and so on. I saw later how Harms succeeded in helping a *Hydroides pectinata* to fix its tube to the substratum by ablation of the abdomen. I wonder if the Pomatoceros could have resisted this drastic measure ?

However this may be, it is seen how the conditions described contribute to the failure of real tube regeneration ; by real is meant a tube that covers completely the worm and allows it to continue living. Observation of the larvæ might reveal in this connexion very interesting details.

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The Larvæ of the Plymouth Galatheidæ.
II. *Galathea squamifera* and *Galathea intermedia.*

By

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With one Figure in the Text and Plate I.

In a previous paper (Lebour, 1930) the general characters of the larvæ of Galathea were described and the various stages of *Galathea strigosa* and *Galathea dispersa* identified. The present paper deals with the larvæ of *Galathea squamifera* and *Galathea intermedia* which have also been followed through their life histories. This completes the larvæ of the Galatheidæ from Plymouth with the exception of *Galathea nexa*, the larvæ of which have not been seen. As is noted in the previous paper this Galathea is regarded as a separate species from *G. dispersa*.

The four Galathea larvæ now known are quite easily distinguishable from one another; firstly, by their size, *G. strigosa* being the largest, *G. dispersa* next, *G. squamifera* smaller still, and *G. intermedia* the smallest of all; secondly, by their colour, notes of which are given below for the two last, those of the two first having already been described; thirdly, by their abdominal spines, *G. squamifera* having them on the fourth and fifth segments, but much smaller than in *G. strigosa*, and *G. intermedia* having them on the fifth segment only as in *G. dispersa*, but the size and colour of the little larva make it unmistakable.

The two larvæ dealt with in this paper have already been partly described by Sars (1900) who takes all the species together, figuring certain stages, but it is not easy to distinguish the two from his figures. Webb (1921) also figures the first larva of *G. squamifera*.

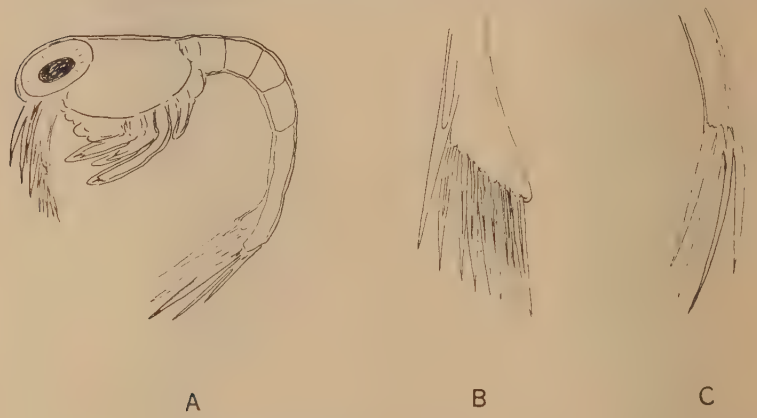
Galathea squamifera is the common inshore form and is usually to be found between tide marks under stones. The larvæ are common inshore, sometimes but rarely, outside, in spring and summer and less commonly in autumn and winter. Females in berry from February onwards. *Galathea intermedia* is not so common, occurring in the Sound and outside on the Mewstone and Eddystone grounds. Larvæ both inside and outside, not so common as *G. squamifera*, chiefly in spring and summer and usually outside the Sound.

Galathea squamifera Leach.

(Plate I, Figs. A–E.)

Four larval stages (no fifth seen), the fourth changing to a young Galathea. First larva from egg, second from first, third from second from plankton, fourth from third from plankton, young Galathea from fourth from plankton. Eggs 0·52 mm. by 0·48 mm. when about half-way to hatching, 0·56 mm. by 0·64 mm. when ready to hatch. At first orange-red, then brownish. Embryonic cuticle like that of Munida with seven long spines on the telson, the antenna with eight spines, the antennule with four (Text-fig. 1, A–C).

Larvæ very transparent, but with much orange-red on the thorax,



Text-Fig. 1.—A, prezoea of *Galathea squamifera*.
B, antenna of same.
C, antennule of same.

first and fifth abdominal segments (sometimes on the second), on the maxillipedes, antennular peduncle (sometimes absent), in front of the eyes (sometimes absent) and at the base of the telson (later this colour is at the hind end of the sixth abdominal segment). Usually, but not always, there is dark brown pigment in three masses at the base of the telson ; in later stages these are on the sixth abdominal segment, above the red. The brown is in the form of three chromatophores which may be contracted or spread out into masses, one behind the other, two of which are on a level in front of it. These brown chromatophores are usually found in the young stages as well as in the later stages, but cases have been seen when they were absent. In the later stages there is more brown, especially above the thorax, and the fourth spine on the telson (the longest) usually has brown pigment at the base. This is the only species at

Plymouth which has the brown pigment to any extent. The red on the fifth and sixth abdominal segments tends to be crimson. There may be yellow in the thoracic region in the later stages. Length of first larva *ca.* 2·2 to 2·5 mm., second larva *ca.* 3·2 mm., third larva *ca.* 4 mm., fourth larva *ca.* 4·8 mm. The rostrum is moderately long with spicules, but not so long as in *G. strigosa*. The carapace spines and spines on the antennal scales are also shorter. The fourth abdominal segment has a pair of very short lateral spines and the fifth a pair of short spines rather longer than those on the fourth. In both they are much smaller than in *G. strigosa*. Denticulations occur on the hind margin of the second to the fifth abdominal segments, becoming smaller in the later stages. The telson is not so wide as in *G. strigosa*. In the last (fourth) stage there are five or six spines on the inner uropods. The young Galathea which emerged from the fourth larva was very like that of *G. strigosa* and *G. dispersa*, with long rostrum armed with five teeth each side and five principal teeth on the side of the carapace with additional smaller teeth between or behind. The colour of the first young stage is reddish, but with the casting of its skin it is brownish with a distinct white mark on the first legs.

Galathea intermedia Lilljeborg.

(Plate I, Figs. F–J.)

Larvæ partly described by Sars (1900) who figures the last stage and the first young stage. This is much the smallest of all the Galathea larvæ and also the brightest coloured.

Four larval stages (no fifth seen), the fourth changing to a young Galathea. First larva from egg, second not seen, third from plankton, fourth (last) from third from plankton, young stage from fourth from plankton. Eggs 0·4 mm. by 0·32 mm. when about three-parts towards hatching, 0·48 mm. by 0·4 mm. when ready to hatch. At first orange-red, then brownish.

Larvæ very transparent, but very brightly coloured. The only Galathea larva known to have orange-red pigment at the outer corners of the telson. Orange-red on the thorax with bright yellow, orange-red on the antennular base, on the second and fourth abdominal segments (on the fourth turning to crimson) and crimson-red at the base of the telson (in later stages on the sixth abdominal segment. An orange-red chromatophore at each corner of the telson at its outer angles. Yellow on the thorax and over the eyes.

Length of first larva *ca.* 2 mm. or less, second not seen, third *ca.* 2·8 to 3·6 mm. (varying in size considerably), fourth *ca.* 3·8 mm. The rostral spine is comparatively short, not always pronouncedly prickly, the carapace spines and spines on the antennal scales and uropods are also

short. Usually there are no lateral spines on the fourth abdominal segment but traces of them may sometimes be seen in the first stage. A pair of short lateral spines on the fifth segment. The outer uropods in the last stage with 7 spines. The first young stage is much like those of the other three species but slightly smaller. It has a fairly long rostrum with three principal teeth and two smaller each side and five lateral teeth on the carapace with one or two smaller intermediate teeth. Behind the rostrum are the characteristic two spines of the adult. The young Galathea is a brilliantly speckled orange-red with bright blue lines in the second stage.

It is now possible to make a key of the larvæ of the Plymouth Galatheidæ :—

I. Antennal scale aciculate : two dorsal spines on the abdominal segments 3, 4, and 5—(Munida).

II. Antennal scale fairly broad, small denticulations on the abdominal segments 3, 4, and 5—(Galathea).

A. Spines on fourth and fifth abdominal segments.

a. larvæ large, first larva *ca.* 3·5 mm., rostrum long, smooth, abdominal spines large—*Galathea strigosa.*

b. larvæ of moderate size, first larva *ca.* 2·2–2·5 mm., rostrum fairly long, prickly, abdominal spines of moderate size—*Galathea squamifera.*

B. Spines on fifth abdominal segment only.

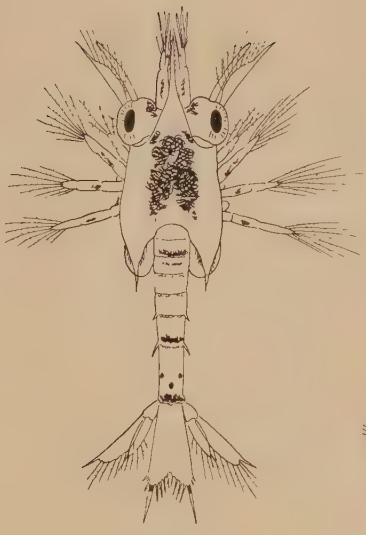
a. larvæ of moderate size, first larva *ca.* 2·6 mm., last larva *ca.* 5 to 6·5 mm. (fourth and fifth). No pigment on angles of telson—*Galathea dispersa.*

b. larvæ small, first larva *ca.* 2 mm. or less, last larva *ca.* 3·8 mm. Orange-red pigment on angles of telson—*Galathea intermedia.*

PLATE I.

FIG. A. *Galathea squamifera* from egg, 2·2 mm. long.
B. „ „ second larva from first from plankton, 3·2 mm. long.
C. „ „ third larva from second from plankton, 4 mm. long.
D. „ „ fourth larva from third from plankton, 4·8 mm. long.
E. „ „ carapace of first juv. from fourth larva.
F. *Galathea intermedia* from egg, 2 mm. long.
G. „ „ third larva from plankton, 2·8 mm. long.
H. „ „ fourth larva from third from plankton, 3·8 mm. long.
J. „ „ carapace of first juv. from fourth larva.

A



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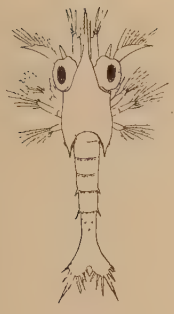

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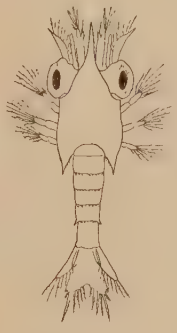

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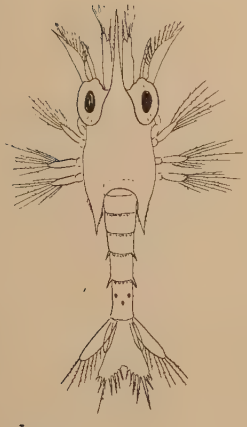

C

J

H

LITERATURE.

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The Vertical Distribution of Marine Macroplankton. X. Notes on the Behaviour of Sagitta in the Plymouth Area.

By

F. S. Russell, D.S.C., B.A.,

Naturalist at the Plymouth Laboratory.

With 8 Figures in the Text.

The researches on the vertical distribution of plankton in the sea in the Plymouth area are tending to indicate that light is a controlling factor of great importance in the behaviour of the larger plankton animals (**13** and **16**). It appears probable that for some species there are certain conditions of light that members of that species tend to collect in during the daytime, and owing to the absorption of light penetrating from above the animals move vertically upwards or downwards towards their light optimum. But in the sea we do not find that *all* the members of the species are concentrated at the level at which apparently the optimum conditions occur ; rather is it that only the greatest number are found at that level, the remainder being at varying distances above and below and decreasing in number with distance apparently as modified by the rate of light absorption. In other words, the animals appear to be distributed around the optimum on a probability curve.

Now in speaking of the members of a species above, it is assumed that we are referring to a population of animals all of the same sex and age. It is well known that animals of different ages seem to prefer different depths (**16**, p. 239) ; therefore in attempting to unravel the leading principles in the behaviour of plankton animals by means of field collections it is essential that we should be careful to base our conclusions on populations of a species all as nearly as possible of the same age. From this standpoint the Crustacea are probably the best group to work on, as the successive stages are well marked and easily recognisable on account of the habit of moulting. It is probably for this reason that of all the animals studied in our previous researches on vertical distribution the copepod *Calanus finmarchicus* has given us the picture of a typical daytime distribution most nearly corresponding to that we should expect if the animals are distributed round an optimum light condition with upper and lower limits within which the population lives, as in the theory previously outlined (**13**,

p. 419) ; for we have known that we were dealing with animals of the same sex and of as nearly as possible the same age.

With the majority of other animals, however, the vertical distributions given in the previous publications have been, for each species, those of populations of a heterogeneous mixture of ages and perhaps of sex. It is not legitimate therefore to attempt to draw fundamental conclusions from such results ; they have merely provided us with a knowledge of the depths at which the majority of any one species live and the range within which a mixed population of that species remains. This important point is well brought out in the following pages in which the behaviour of a Sagitta population is dealt with.

General Results.

The details given here represent the results of collections made with the 2-metre stramin ring-trawl during 24 hours on two different occasions, the first on July 15–16th, 1924, and the second on June 3–4th, 1926.

A careful examination of the Sagitta occurring on these occasions has shown that there are in the Plymouth area two common species, *Sagitta elegans* Verrill, and what is probably *Sagitta setosa* J. Müller. These two species have in previous records been included under one name, *Sagitta bipunctata* Quoy and Gaimard, a name now used for a warm-water species apparently not occurring in this area (**17**, pp. 7–16, and **18**, p. 19). The results for the Sagitta population as a whole on June 15–16th, 1924, have already been published (**11**, p. 792)—under the name of *Sagitta bipunctata ;* the original material has since been re-examined and studied in more detail and the results are given in the following pages. Fortunately it was found that on that date almost the entire catches were made up of *S. elegans*, and that *S. setosa* was either absent or only present in such small numbers as not to affect the general picture given in **11**, page 792 and Figure 3, which must now be regarded as referring to *S. elegans*.

On June 3–4th, 1926, *S. setosa* was much more common, and it is probable that its abundance varies from year to year. An examination of the Sagitta populations throughout the year 1930 has shown that *S. setosa* was almost absent in June and July. Meek (**8**, p. 743) has found in the North Sea off the Northumberland coast that the two species, *S. elegans* and *S. setosa*, vary much in their relative abundance, the years in which one species was common apparently showing a rarity of the other species and vice versa.

The collections described in this paper were both made with the 2-metre stramin ring-trawl and the full conditions at the time of collecting have already been published (**11** and **15**). On each date five series of collections were made at definite times during the 24 hours, the net being towed horizontally for periods of ten minutes each at different depths in each

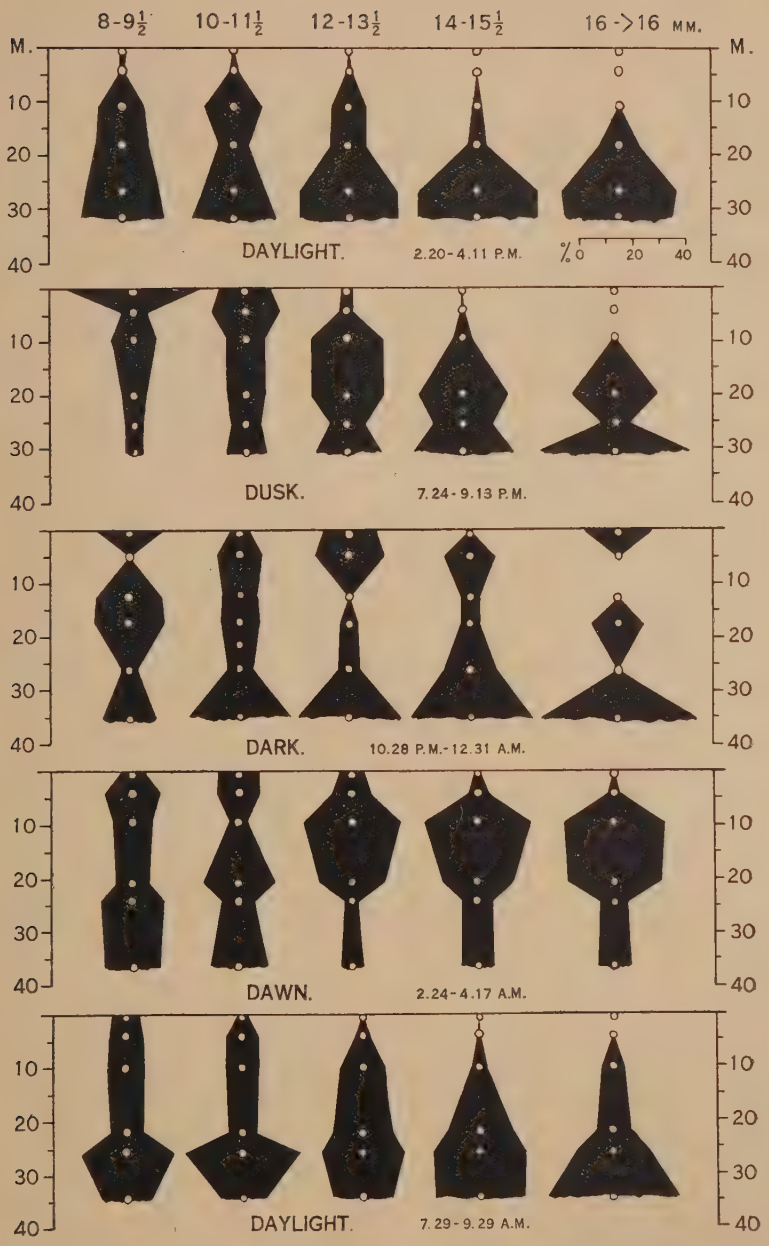

FIG. 1.—The percentage vertical distribution of *Sagitta elegans* at the times shown on June 3rd–4th, 1926. For each period—" daylight," " dusk," " dark," " dawn," and " daylight "—the Sagitta are divided into 8–9½, 10–11½, 12–13½, 14–15½, and 16–>16 millimetre groups. The white spots and black circles indicate the average depths at which hauls were taken. Bottom was at 54 metres.

series, the depths being indicated by the depth-recorder (**11**, Fig. 1, and **15**, Fig. 1).

Measurements were made of the total lengths of Sagitta from samples from each collection ; the measurements were made to the nearest half-millimetre, but have here been divided into 2-millimetre groups. The numbers in each 2-millimetre group of those measured have been given in Tables IV, V, and VI on pages 409–411 ; unfortunately in the June, 1926, collections the numbers measured are on some occasions rather low. The collections had been previously worked up and samples only of the whole catches kept ; on finding that there were two species present it was necessary to re-examine the samples, and as a result of dividing the Sagitta into the two species the numbers for each species measured are low. The indications of the behaviour of the animals on this occasion appear however to be quite definite and are confirmed by the July, 1924, collections for which larger numbers of animals were available for measurement.

Tables IV–VI show the numbers measured in each sample, and in Tables VII, VIII, and IX (pp. 412–414) are given the calculated total numbers of Sagitta in each 2-millimetre length group for each collection. These results, given in Tables VII–IX, are expressed diagramatically in Figures 1, 2, and 3, where the vertical distribution diagrams are given for each length group, the numbers at each depth in each diagram being expressed as percentages of the total number at all depths for that diagram.

These diagrams show that in the daylight the younger stages of *Sagitta elegans* are apparently able to withstand much higher intensities of light than the older stages, and can also live within a wider range of intensities. This change in depth with age has already been shown for other species, e.g. *Krohnia hamata* (Fowler, **4**, p. 76). At dusk it seems that the youngest stages, which were living high in the water in the daytime, have reached the surface in numbers while the older stages are still deep down. This behaviour is most clearly brought out in Figure 1, which shows the behaviour of *S. elegans* on June 3–4th, 1926. A very similar type of behaviour was shown on July 15–16th, 1924 (Fig. 2), but in this figure there are no individuals over $13\frac{1}{2}$ mm. in length. In comparing Figures 1 and 2 this latter point should be borne in mind and only the diagrams for similar size groups compared one with another. After dark the numbers caught in 1926 were really too low to give significant results ; in 1924, however, there is an indication that all stages were to be found unevenly mixed from top to bottom.

In the dawn a process of resorting is taking place, and on both occasions it is very evident that the older stages started to leave the surface first ; this is clearly shown in both Figures 1 and 2, while in Figure 1 the stages from 12 mm. and upwards in length appear already to be massing in an

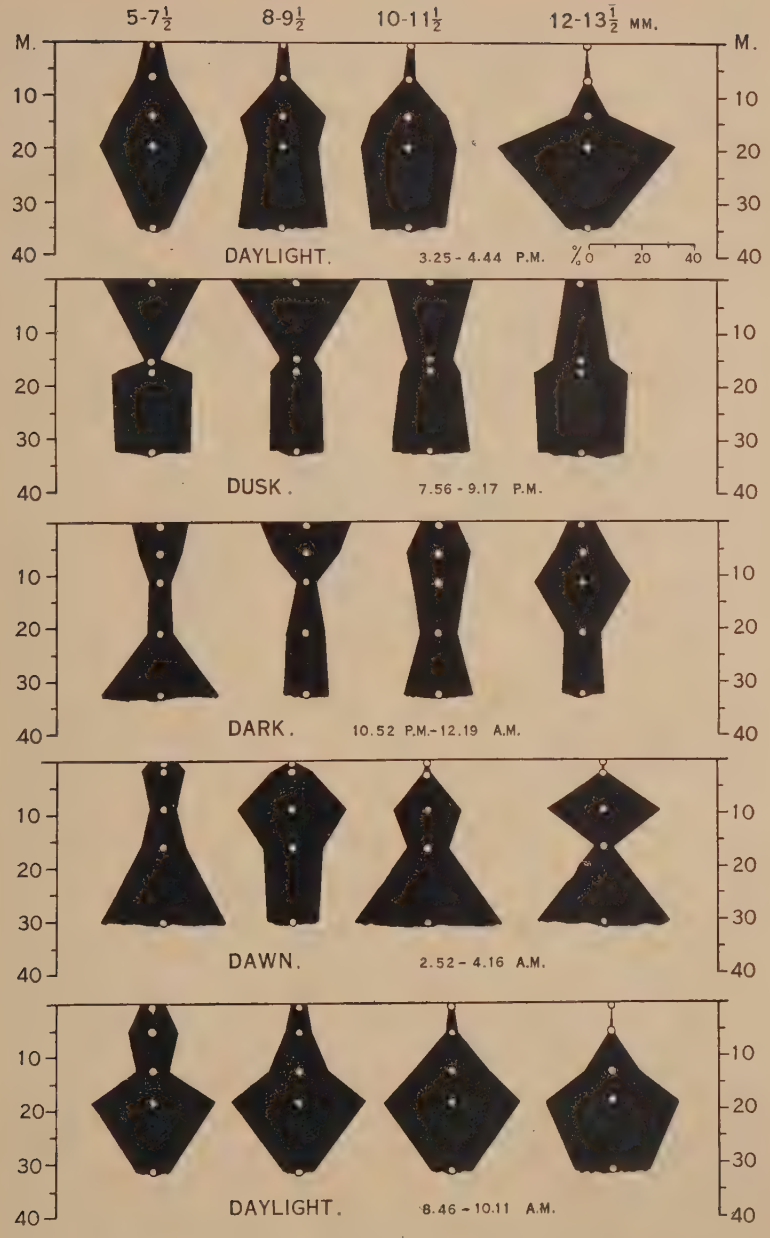

FIG. 2.—The percentage vertical distribution of *Sagitta elegans* at the times shown on July 15th–16th, 1924. For each period—"daylight," "dusk," "dark," "dawn," and "daylight"—the Sagitta are divided in 5–7½, 8–9½, 10–11½, and 12–13½ millimetre groups. The white spots and black circles indicate the average depths at which hauls were taken. Bottom was at 54 metres.

optimum region. The following day the vertical distribution has assumed a picture very similar to that of the previous afternoon, except that there is a tendency for the Sagitta to stretch more into the upper layers—a phenomenon exhibited by other plankton animals on that day and probably due to clouding of the water by a thick swarm of Calanus.

On June 3–4th, 1926, *Sagitta setosa* were on the whole not sufficiently abundant to give a picture of their behaviour ; at dusk, however, when they were most abundant in the catches, their behaviour appears to have been very similar to that of *S. elegans*. The younger stages have migrated to the surface before the larger forms, as shown in Figure 3. It is to be noticed also that on the whole there is a tendency for all stages to be

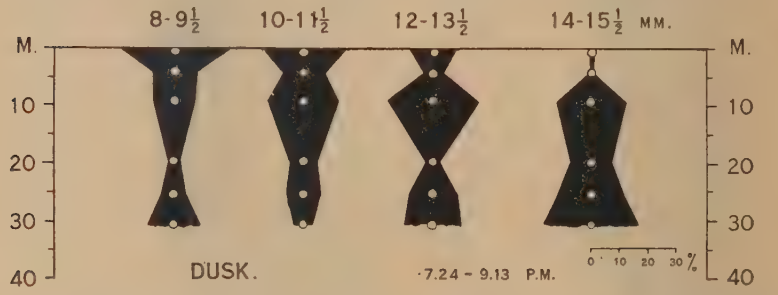

Fig. 3.—The percentage vertical distribution of *Sagitta setosa* at " dusk " on June 3rd, 1926. The Sagitta have been divided into 8–9½, 10–11½, 12–13½, and 14–15½ millimetre groups. The white spots and black circles indicate the average depths at which hauls were taken. Bottom was at 54 metres.

slightly higher in the water than the corresponding stages of *S. elegans* at the same time (cf. Fig. 1) : whether this difference is shown also in the daytime we cannot say until further observations have been made.

Huntsman in the Atlantic waters of Canada gives indications of a similar type of behaviour with *Sagitta elegans*. For instance (**5**, p. 465), he divided the Sagitta into two groups, those under and those over 20 mm. in length. Of the larger group specimens were only obtained at the surface at 6 p.m., 9 p.m., and 12 midnight ; while those under 20 mm. were present at the surface at all hours of the day and night, and there is an indication that the smallest sizes stay nearer the surface longest in the daytime, e.g. 9 a.m., occasional specimens up to 10 mm. long ; 12 noon, occasional specimens up to 9 mm. ; 3 p.m., many specimens up to 13 mm. ; 6 p.m., very many specimens up to 16 mm. ; 12 midnight, many specimens up to 20 mm. ; 3 a.m., several specimens up to 18 mm. ; and 6 a.m., occasional specimens up to 11 mm. He says further, " inside the Gulf in June larger individuals came to the surface than came above 30 metres in August, for 9 a.m., 12 m., 9 p.m., 12 midnight and 6 a.m. This decrease

in the daily vertical migration is doubtless due to the warming of the surface water."

He sums up, "The facts point to the following conclusions: *Sagitta elegans* is confined to water of comparatively low salinity, being stopped in its migration into the depths by water of high salinity. It is affected by light, coming nearer the surface at night. It is affected by temperature, keeping to the colder water. The young behave differently from the adults, living in the lighter, warmer surface water. With increasing age it becomes gradually restricted to the darker, colder water, which is deeper." These results are confirmed by Bigelow (**2**, p. 316) in his survey of the plankton of the Gulf of Maine, and Huntsman and Reid (**6**) give further data on increase of size with depth.

A Comparison of the Behaviour of Sagitta with the Light Intensity Conditions.

In a previous publication I illustrated in outline the theory of the vertical movements of plankton animals on the basis that they were distributed in a chance distribution around an optimum condition of light in the daytime. It was suggested that they followed this optimum condition towards the surface at dusk, and that at night the light stimulus was removed and they became free to move anywhere.* The following day those near the surface pick up their optimum condition again and follow it downwards as the light increases in strength, picking up recruits from deeper layers as they come into the influence of the stimulus of the increasing light. "It seems most probable that the types of distribution shown by the different species are due to a combination of the speed of upward movement of which they are capable and the time at their disposal for such upward migration" (**16**, p. 236).

This theory appears essentially to be borne out by the results here given for *Sagitta elegans;* we see very clearly how at dusk the younger stages only have had time to reach the surface *en masse* before the light fades away, but the older stages coming from deeper layers never have time to reach the surface in quantity until after dark when they are free to roam anywhere.

An attempt has been made to compare these results with the actual variations of the light intensity beneath the surface of the sea. Throughout the year 1930 continuous records of light intensity have been made from a photometer on the parapet of the flat roof of the Laboratory. The photometer contained a Burt vacuum sodium photo-electric cell sensitive mainly to blue light, and the intensities were recorded on a thread recorder

* It must be understood that this behaviour would be modified under extreme conditions of other factors, such as temperature, as would the vertical movements (Russell **13**, p. 435, and Nikitine **9**).

in the Laboratory (**1**). It so happened that on June 4th, 1930, the weather was cloudless and clear, and the light conditions must have approximated very closely to those on June 3rd and 4th, 1926, when the collections of Sagitta were made (for weather conditions see **15**, p. 830). The actual light intensities throughout June 4th, 1930, have been recorded on the above instrument, and I am able here to publish the curve (Fig. 4) through the kind permission of Dr. W. R. G. Atkins by whom the researches on light intensity are being carried out. In Figure 4 the actual intensities are given as thousands of metre candles, based upon a standardization of a vacuum potassium cell against a carbon arc ; the sodium cell was then standardized in daylight against the potassium cell.

Unfortunately, however, we do not know the transparency of the water

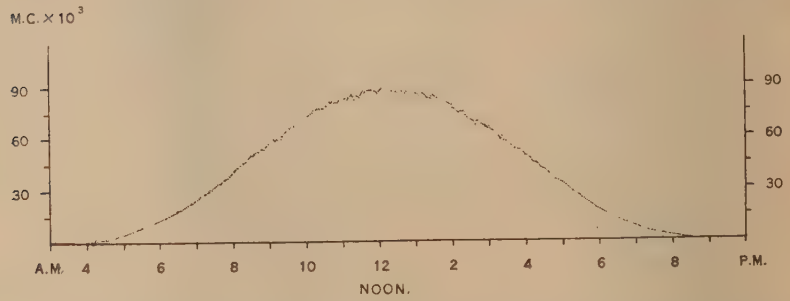

Fig. 4.—Curve of light intensity obtained on roof of Plymouth Laboratory with vacuum photo-electric cell and thread-recorder (**1**) on June 4th, 1930 : a clear cloudless day. (By courtesy of Dr. W. R. G. Atkins.) The intensities are given as thousands of metre-candles.

on June 3rd, 1926, except that the Secchi disc disappeared at a depth of 10 metres, and we are therefore unable to estimate for certain the intensities at different depths beneath the surface. Animal plankton on that day was very abundant, and it is possible that the coefficient of absorption may have been in the region of 0·200 ; it seems almost certain to have been considerably more than 0·100. Poole and Atkins (**10**, p. 308) give as a mean coefficient of absorption for Station E1 for a number of readings throughout the year 0·150 for the upper 20 metres, 0·120 for 20 to 40 metres, and 0·111 for 40 to 60 metres. Moreover, their work has shown that there is considerable variation to be found in the opacity of the water at different depths on any one day, due possibly to the aggregation of plankton animals into definite zones. One cannot therefore hope to estimate with any accuracy the light conditions beneath the surface unless actual light measurements have been made, at any rate at a few depths.

Taking a coefficient of absorption of 0·200 for all depths with a surface loss of illumination of 15% by reflection (**10**, p. 309) and using the air

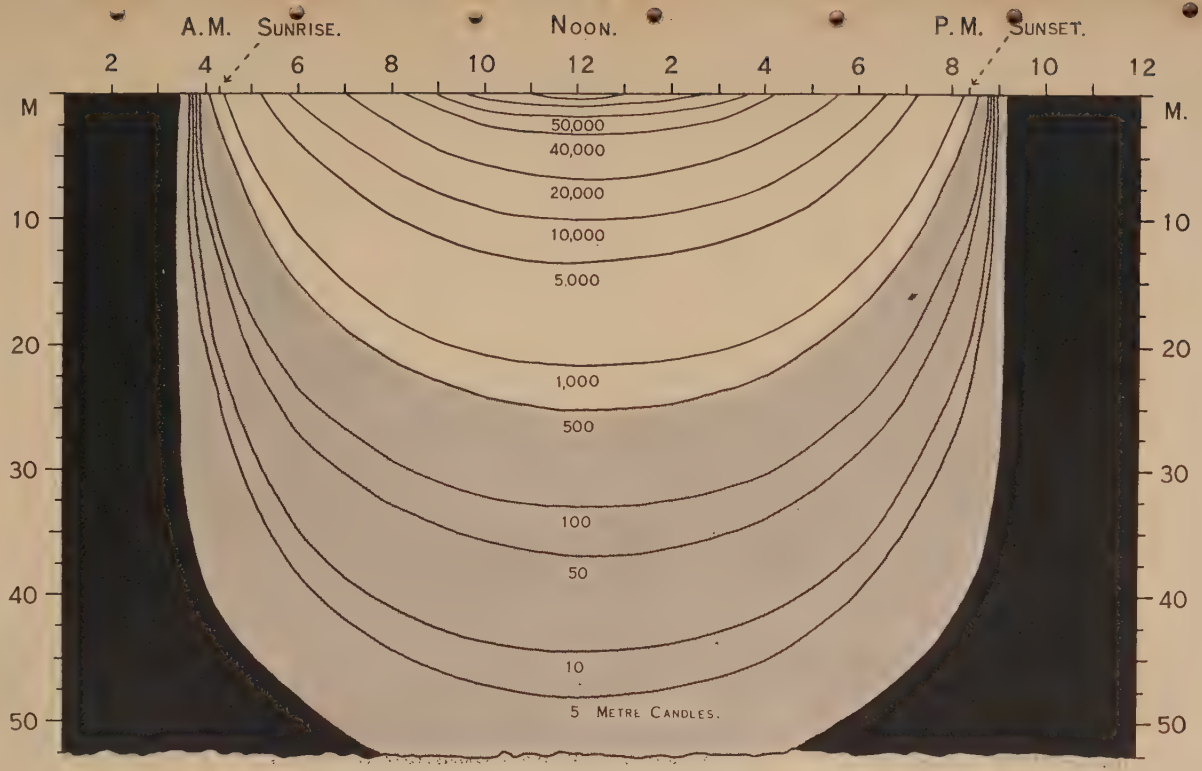

Fig. 5.—Curves of equal light intensities beneath the sea surface throughout the 24 hours, calculated from the air intensities obtained on June 5th, 1930, taking the coefficient of absorption of the water as being 0·200 at all depths and allowing for a reflection loss of 15%. The blackened area corresponds to the zone of "darkness" (see text) and the stippled area to the region in which "twilight" intensities would be experienced. The intensities are given in metre-candles and the depths in metres.

illumination figures obtained for June 4th, 1930 (Fig. 4), I have calculated the intensities beneath the sea surface at all times of the day.* By means of these results I have drawn in Figure 5 the lines of equal light intensities beneath the sea surface throughout the day. As I have already said, this picture can have no real significance and must be hypothetical only, but it is instructive as giving an idea of the conditions beneath the sea surface and as we shall see later it will serve a purpose. In Figure 7, p. 403, I have also given the lines of equal intensities for the same conditions, save that in this case the coefficient of absorption has been taken as being 0·100 at all depths. It is probably somewhere between the conditions figured in Figures 5 and 7 that the conditions beneath the surface on June 3rd and 4th, 1926, lay. In both these figures I have marked in black the area in which the conditions are those of "darkness" in the accepted meaning of the word, that is where the light intensity is such as we experience well after sunset when the stars are shining brightly, or less. The shaded area represents intensities of light lying between darkness and the illumination almost immediately after sunset on a clear evening, conditions which would be covered by the term "twilight." We thus can see at a glance at what times and depths darkness or twilight conditions would be experienced.

Let us study first Figure 5. It shows that between the hours of 7 a.m. and 5 p.m. the lines of equal intensities do not vary greatly in depth, but that before and after these times their slopes become very steep until eventually the light fades out almost abruptly. From this figure we could easily understand how animals living around an optimum of, say, 5000 m.c. at midday will have time to mass at the surface in the evening if they follow their optimum upwards; but animals which live at 50 m.c. at midday will be unable to keep pace with the suddenly disappearing light at evening and will never reach the surface in great numbers as the light stimulus is removed too soon.

But if we compare this figure with the behaviour of Sagitta given in Figures 1 and 2 an interesting point arises. In Figure 6 I have plotted the depths at which *Sagitta elegans*, 12 to 13½ mm. in length, first appear beneath the surface in abundance equivalent to 30% on the scale given in Figures 1 and 2, in the morning and afternoon of the two days in question.

* I am indebted to Dr. W. R. G. Atkins for the light intensities after sunset. It is probable that actually at sunset the usual illumination is in the neighbourhood of 1000 metre candles: the figures given me by Dr. Atkins were measured on October 3rd, 1927, and were as follows: sun just down, 620 m.c.; 15 minutes after, 530 m.c.; 20 minutes after, 178 m.c.; 26 minutes after, 73 m.c.; 30 minutes after, 35 m.c. (small print readable); 34 minutes after, 19 m.c. (small print readable with difficulty); 39 minutes after, 9·5 m.c.; 41 minutes after, 2·5 m.c. (Jupiter and first magnitude stars visible). The lag between sunset and 15 minutes after was probably due to cloud reflection: it has been smoothed out in Figures 5 and 7. The intensities before sunrise are taken as being the same as those after sunset.

It will be seen that on June 3rd–4th, 1926, the two points lie very close to 25 metres, and on July 15th–16th, 1924, they again appear very nearly at the same depth, namely, in this case 15 metres. From the curves of equal intensities given in Figure 5 it appears that in 1924 the Sagitta were therefore becoming abundant at somewhere between 2000 and 3000 metre-candles in both the morning and the afternoon, and similarly that in 1926 these points lay somewhere between 300 and 400 m.c. If now we plot the similar points for the dawn distribution we find that in 1924 it lies not on the 2000–3000 m.c. curve, but only at 50–100 m.c., at about

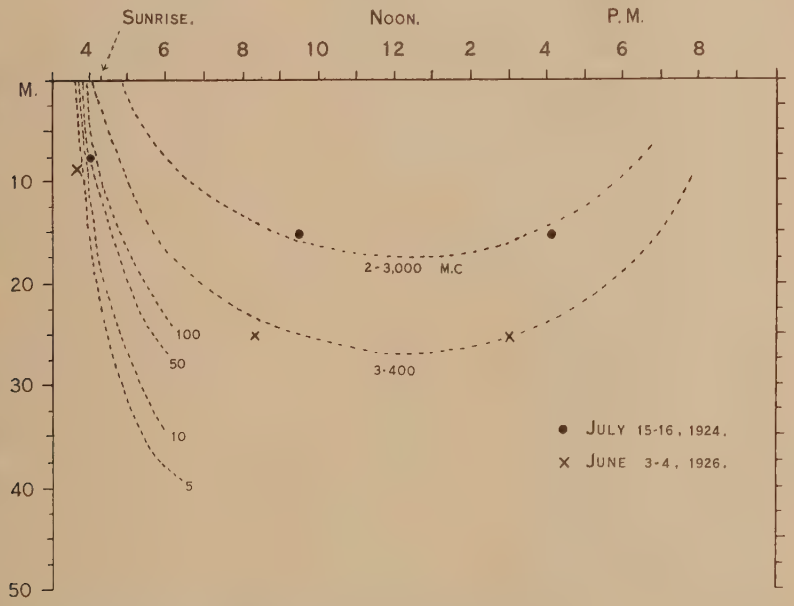

FIG. 6.—Depths at which *Sagitta elegans* of 12 to 13½ mm. in length first appear beneath the surface in an abundance equalling 30% on the scale in Figures 1 and 2 on June 3rd–4th, 1926, and July 15th–16th, 1924, in the morning, afternoon, and at "dawn." The dotted curves are the lines of equal light intensities taken from Figure 5.

7·5 metres. A similar state of affairs is shown for 1926, where the 30% point lies at about 8·5 metres at an intensity of below 5 m.c. Now the close agreement in the intensities at which the Sagitta are living in the morning and afternoon may be mere coincidence and have no significance, since we cannot say for certain what the intensities were on the days in question. But the difference between the intensities to which the Sagitta were reacting at dawn and those which they experienced in the daytime must be significant. Examination of Figures 1 and 2 shows clearly that the Sagitta of 12 mm. and over had started to leave the surface even before sunrise,

For instance, on June 4th, 1926, Sagitta 12–13½ mm. long were already showing signs of leaving the surface, and those of 14–15½ mm. in length and longer had already left the surface layers down to 4 metres when the haul at that depth was made at 3.49–3.59 a.m. : this is well before sunrise, which occurred at 4.17 a.m.,* and the air illumination must probably have been less than 300 m.c. at any rate. Similarly, on July 16th, 1924, Sagitta 12–13½ mm. long had already left the surface layers by 3.51–4.1 a.m., when sunrise should be at 4.24 a.m. (sunrise not recorded in log).

It seems from these data that the Sagitta must have started to leave the surface before the illumination reached that at which many are found to be living in the daytime. This would appear to indicate that the Sagitta are more sensitive to light immediately after the darkness of the night than they are later in the daytime. Thus is would seem that they can become adapted to light conditions of higher intensity as the morning goes on.

Indications that this may be so with some fresh-water plankton animals is given by Worthington, who says (**19**, p. 2) as a result of observations made during 26 hours in Lake Lucerne, " Most species start their descent at dawn and reach their lowest level before the sun attains its zenith. Then a slow upward movement starts, accelerates at dusk, and continues for two or three hours after complete darkness."

Clarke, experimenting with the fresh-water Daphnia, has recently produced evidence that this crustacean can become adapted to light of various intensities. He suggests that (**3**, p. 120) " There is, then, no ' absolute optimum ' light intensity for these *Daphnia*. They do not seek any particular intensity of illumination. The animals become adapted to the light intensity which exists at that time and place—this is for them a ' relative optimum.' "

That plankton animals should show a certain power of adaptation to light conditions is probably to be expected, and it would seem that possibly they live around a shifting optimum during the course of the day. It should be borne in mind, however, that probably they are only capable of adaptation within limits, and that outside these limits definite avoiding reactions set in. Until observations in the field are made synchronously with accurate light-intensity measurements at all depths it is premature to discuss the matter further.

Figure 7 has been inserted to draw attention to the very great effect that the transparency of the water has upon the intensity of the illumination beneath the surface. In this figure the coefficient of absorption of the water has been taken as 0·100 at all depths, and it can be seen that each iso-intensity line has been pushed down exactly twice as deep as in Figure 5,

* This is the sunrise time recorded in the log : theoretically it should have been 4.11 a.m. : the discrepancy is probably due to " sunrise " being the appearance of the sun above the hills of Dartmoor.

where the coefficient of absorption was taken as being 0·200 at all depths. This will mean that animals living in the "twilight" zone with an upper limit of about 500 m.c. will be abundant nearly up to 25 metres at midday under the conditions of Figure 5, while under the conditions of Figure 7 they will be forced right to the bottom.

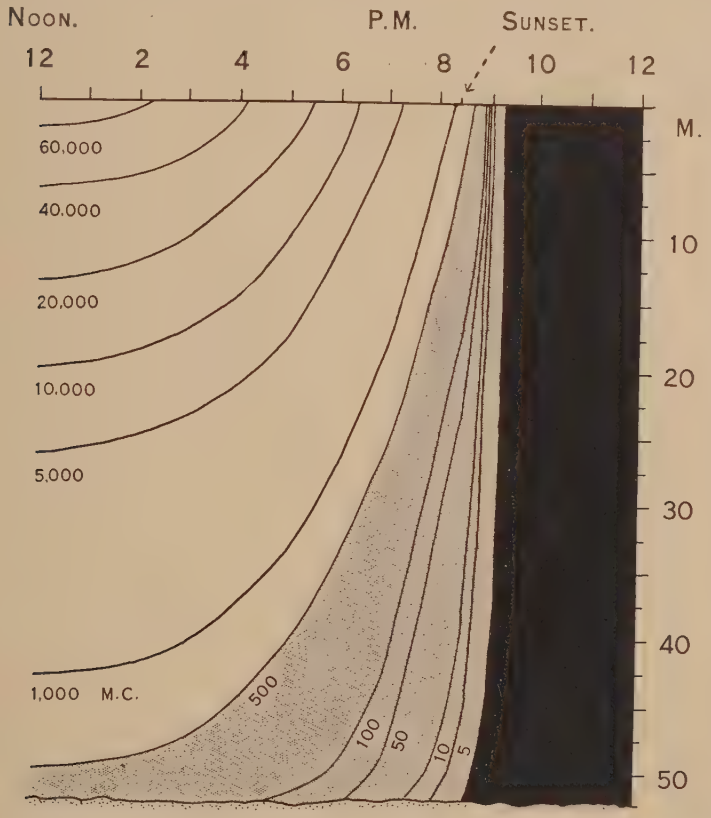

FIG. 7.—Curves of equal light intensities beneath the sea surface from noon to midnight, calculated from the air intensities obtained on June 5th, 1930, taking the coefficient of absorption of the water as being 0·100 at all depths and allowing for a reflection loss of 15%. The blackened area corresponds to the zone of "darkness," and the stippled area to the region in which "twilight" intensities would be experienced. The intensities are given in metre-candles and the depths in metres.

In view of the above I have reproduced here Figure 8 which gives the behaviour of Sagitta on two successive days, June 16th, 17th, and 18th, 1925. This figure has already been published as for *S. bipunctata* (**13**, p. 88); unfortunately the material is no longer available, so that it is not

possible to say whether *S. elegans* or *S. setosa* predominated. It can be seen that the behaviour here shown differs very markedly from that on the other two dates given in Figures 1 and 2 in this present publication. On June 16th–18th, 1925, the Sagittas became very abundant at night, but in the daytime they were very scarce, and the impression gained is that they must have retreated to very near the bottom (54 m.) in the daytime. It may well have been that on these two days in June, 1925, the water was far more transparent than on the two occasions in 1924 and 1926 ; certainly the animal plankton was very much less abundant, on the two days in 1925, than it was on either of the days in 1924 or 1926, and this alone would probably have an appreciable effect on the transparency of the water. But why the Sagitta should have left the surface so suddenly

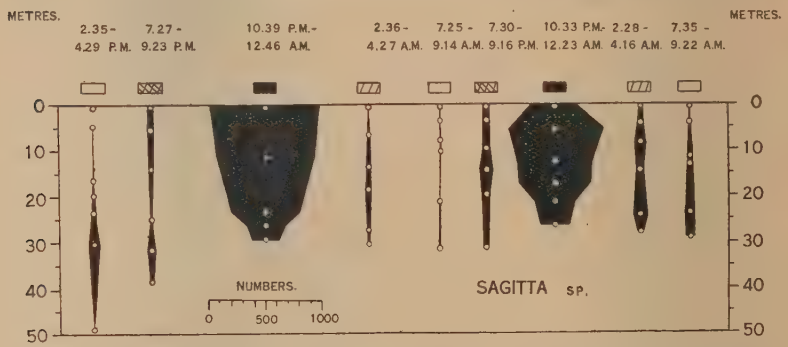

FIG. 8.—The vertical distribution of Sagitta sp. at the times shown, on June 17–18–19th, 1925. The plain, cross-hatched, black, and shaded rectangles represent " daylight," " dusk," " dark," and " dawn " respectively. The white spots and black circles indicate the average depths at which hauls were taken.

in the early mornings of June 18th and 19th, 1925, is hard to explain. Inspection of Figure 8 shows that apparently the majority of the Sagitta had already left the upper 30 metres by 2.36 a.m. and 2.28 a.m., about an hour and a half before sunrise. At this time the light intensity must have been almost negligible and some other factor was perhaps operating.

It should be realised that on days of intermittent sunshine the light intensity beneath the sea surface is changing to an enormous degree. Under such conditions it is natural to suppose that the animals take up an average position in the water, being constantly stimulated to move upwards or downwards. On clear bright days, however, the position in the water appears to be very constant, but presumably it must take a little time for a population of animals to settle around their optimum conditions.

This leads one to wonder whether in nature there are not perhaps two

separable types of behaviour, namely the picking up of optimum conditions by means of random movements under fairly constant intensities, and the stimulation of active responses by sudden changes in intensity. This latter would operate at dusk and dawn; that such a behaviour is possible is perhaps indicated by the work of Kikuchi (7) who has shown an apparent double migration to the surface in the evening, first on the sun striking behind a mountain and secondly at the time of true sunset. This might possibly account for the sudden disappearance of Sagitta so early before sunrise.

SUMMARY.

1. The diurnal behaviour of Sagitta of different sizes is shown as a result of collections made with the 2-metre stramin ring-trawl throughout 24 hours on July 15th–16th, 1924, and on June 3rd–4th, 1926.

2. The younger stages of *Sagitta elegans* appear to withstand higher intensities of light in the daytime than do the older stages, and they migrate first to the surface at dusk, as do those of *Sagitta setosa*. The older stages leave the surface first at dawn.

3. Diagrams are given showing the curves of equal intensities of light beneath the sea surface throughout 24 hours as calculated from air intensities obtained in June, with a reflection loss of 15%; (*a*) assuming a coefficient of absorption of 0·200 at all depths, and (*b*) assuming a coefficient of absorption of 0·100 at all depths.

4. It appears, from a comparison of the behaviour of *Sagitta elegans* with the calculated light conditions beneath the surface, that the older Sagitta at any rate are more sensitive to light in the early morning after the darkness of the night and can become adapted to light of higher intensities later in the day.

5. Previously published observations on the behaviour of Sagitta sp. on June 17th–18th–19th, 1925, show quite a different type of behaviour from the above, possibly due to more transparent water, the Sagitta only appearing abundantly above 30 metres in the dark. They had again migrated to below 30 m. before there was any appreciable light.

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TABLE I.

Total numbers of Sagitta elegans caught in 10-minute hauls of the 2-metre Ring-trawl at each depth on June 3rd–4th, 1926.

	Daylight. 2.20–4.11 p.m.	Dusk. 7.24–9.13 p.m.	Dark. 10.28 p.m.–12.31 a.m.	Dawn. 2.24–4.17 a.m.	Daylight. 7.29–9.29 a.m.
S.	40	513	143	257	117
II.	92	392	188	454	347
III.	1028	755	108	1152	852
IV.	1117	1318	156	993	1426
V.	3784	766	124	388	2428
VI.	4090	1530	456	507	2112

TABLE II.

Total numbers of Sagitta setosa caught in 10-minute hauls of the 2-meter Ring-trawl at each depth on June 3rd–4th, 1926.

	Daylight. 2.20–4.11 p.m.	Dusk. 7.24–9.13 p.m.	Dark. 10.28 p.m.–12.31 a.m.	Dawn. 2.24–4.17 a.m.	Daylight. 7.29–9.29 a.m.
S.	13	677	287	93	13
II.	49	288	452	116	244
III.	92	745	412	338	107
IV.	83	252	264	207	194
V.	167	464	317	172	272
VI.	120	550	604	72	808

TABLE III.

Total numbers of Sagitta elegans caught in 10-minute hauls of the 2-metre Ring-trawl at each depth on July 15–16th, 1924.

	Daylight. 3.25–4.45 p.m.	Dusk. 7.55–9.17 p.m.	Dark. 10.50 p.m.–12.20 a.m.	Dawn. 2.50–4.16 a.m.	Daylight. 8.45–10.11 a.m.
S.	98	2060	1040	66	355
II.	196	1230	1600	203	760
III.	1195	1460	1760	1010	3400
IV.	2925	2290	990	490	7130
V.	1190	2400	1573	1510	2710

TABLE IV.

ACTUAL NUMBERS OF SAGITTA ELEGANS MEASURED FROM CATCHES ON JUNE 3RD–4TH, 1926.

Depth in metres.	Length in millimetres. <7½	8–9½	10–11½	12–13½	14–15½	>16	Total.
DAYLIGHT. 2.20–4.11 p.m.							
S.	1	11	10	14	2	–	38
4·3	–	2	15	10	2	1	30
10·8	1	7	33	45	14	–	100
18	3	7	15	42	24	9	100
26·6	–	3	10	35	44	8	100
31·5	1	3	16	32	41	7	100
DUSK. 7.24–9.13 p.m.							
S.	4	16	15	5	–	–	40
4·4	1	4	25	7	2	–	39
9·3	2	6	12	35	9	–	64
19·7	–	3	11	33	36	17	100
25·3	1	3	10	19	28	7	68
30·3	–	2	10	26	36	26	100
DARK. 10.28 p.m.–12.31 a.m.							
S.	1	4	2	5	–	1	13
4·6	–	–	3	4	3	–	10
12·3	–	4	4	–	2	–	10
17·2	1	5	5	2	2	1	16
25·9	–	1	3	2	5	–	11
35	2	3	10	9	10	2	36
DAWN. 2.24–4.17 a.m.							
S.	–	1	6	5	1	–	13
4	–	4	10	13	8	2	37
9·5	1	3	6	36	48	20	114
20·5	1	3	20	24	30	17	95
24·4	–	5	8	5	11	4	33
36·3	–	4	14	7	12	5	42
DAYLIGHT. 7.29–9.29 a.m.							
S.	–	5	6	2	3	–	16
4	–	4	11	12	3	1	31
10	1	2	5	15	7	4	34
21·8	–	2	5	22	28	6	63
25·7	1	5	19	30	38	14	107
34·3	1	3	8	23	40	26	101

TABLE V.

ACTUAL NUMBERS OF SAGITTA SETOSA MEASURED FROM CATCHES ON JUNE 3RD–4TH, 1926.

Depth in metres.	<7½	8–9½	10–11½	12–13½	14–15½	>16	Total.
			DAYLIGHT. 2.20–4.11 p.m.				
S.	–	2	3	2	3	2	12
4·3	–	5	6	1	4	–	16
10·8	2	2	3	1	1	–	9
18	1	1	3	3	–	–	8
26·6	1	2	5	6	1	1	16
31·5	–	1	5	1	1	1	9
			DUSK. 7.24–9.13 p.m.				
S.	1	22	26	15	1	1	66
4·4	1	8	13	6	1	–	29
9·3	1	8	20	24	11	1	65
19·7	–	2	7	4	6	1	20
25·3	–	5	10	13	11	3	42
30·3	–	10	6	16	16	3	51
			DARK. 10.28 p.m.–12.31 a.m.				
S.	–	2	7	7	5	5	26
4·6	1	1	2	4	16	–	24
12·3	–	5	8	4	14	7	38
17·2	–	4	3	4	12	3	26
25·9	–	2	4	8	9	5	28
35	2	3	3	12	21	7	48
			DAWN. 2.24–4.17 a.m.				
S.	–	1	3	4	–	–	8
4	–	3	1	6	1	–	11
9·5	1	4	5	10	9	4	33
20·5	2	2	4	4	5	3	20
24·4	1	4	5	2	3	1	16
36·3	–	1	–	1	3	–	5
			DAYLIGHT. 7.29–9.29 a.m.				
S.	1	–	–	–	1	–	2
4	2	14	5	1	1	–	23
10	–	1	–	2	3	–	6
21·8	–	1	3	2	3	–	9
25·7	–	2	–	5	6	–	13
34·3	–	3	8	8	12	8	39

TABLE VI.

ACTUAL NUMBERS OF SAGITTA ELEGANS MEASURED FROM CATCHES ON JULY 16–17TH, 1924.

Depth in metres.	<7½	8–9½	10–11½	12–13½	>14	Total.
DAYLIGHT. 3.25–4.45 p.m.						
S.	4	7	16	6	–	33
6·6	12	17	43	29	–	101
13·6	10	34	94	79	7	224
19·5	5	10	44	127	14	200
34·5	2	18	49	43	3	115
DUSK. 7.55–9.17 p.m.						
S.	12	67	123	55	1	258
7	–	–	–	–	–	–*
15·1	1	9	32	47	3	92
17·1	6	16	50	93	8	173
32·3	6	18	73	89	6	192
DARK. 10.50 p.m.–12.20 a.m.						
S.	5	17	23	19	–	64
5·8	4	12	37	39	1	93
11·2	2	4	31	63	2	102
20·7	3	10	31	36	2	82
32·2	11	9	44	34	2	100
DAWN. 2.50–4.16 a.m.						
S.	23	9	7	–	–	39
1·9	37	51	28	5	–	121
9	3	15	26	22	1	67
16	5	9	18	4	–	36
30·1	13	8	62	30	–	113
DAYLIGHT. 8.45–10.11 a.m.						
S.	38	62	34	1	–	135
5·3	12	15	15	3	–	45
12·5	6	23	69	42	1	141
18·3	16	38	90	78	–	222
31·1	7	16	49	81	2	155

* The tube containing these specimens had dried up.

TABLE VII.

TOTAL NUMBERS OF SAGITTA ELEGANS AT 2-MILLIMETRE INTERVALS OCCURRING AT EACH DEPTH; ON JUNE 3RD–4TH, 1926, IN 10-MINUTE HAULS WITH THE 2-METRE RING-TRAWL.

Depth in metres.	Time.	Length in millimetres. <8	8–9½	10–11½	12–13½	14–15½	>15½	Total.
		DAYLIGHT.						
S.	4.1 p.m.	1	12	10	15	2	–	40
4·3	3.44 „	–	6	46	31	6	3	92
10·8	3.25 „	10	71	340	463	144	–	1028
18	3.3 „	33	78	168	469	268	101	1117
26·6	2.42 „	–	113	378	1325	1665	303	3784
31·5	2.20 „	41	123	655	1308	1677	286	4090
		DUSK.						
S.	9.3 p.m.	51	205	195	62	–	–	513
4·4	8.46 „	12	39	251	71	19	–	392
9·3	8.26 „	23	68	143	415	106	–	755
19·7	8.9 „	–	39	145	435	475	224	1318
25·3	7.48 „	15	31	115	215	314	76	766
30·3	7.24 „	–	31	153	397	552	398	1530
		DARK.						
S.	12.21 a.m.	11	45	22	54	–	11	143
4·6	12.1 „	–	–	56	76	56	–	188
12·3	11.36 p.m.	–	43	43	–	22	–	108
17·2	11.13 „	9	48	48	21	21	9	156
25·9	10.51 „	–	11	33	23	57	–	124
35	10.28 „	27	37	128	109	128	27	456
		DAWN.						
S.	4.7 a.m.	–	21	117	98	21	–	257
4	3.49 „	–	50	123	159	99	23	454
9·5	3.29 „	12	35	57	369	484	196	1152
20·5	3.8 „	10	30	209	247	318	179	993
24·4	2.47 „	–	58	93	58	128	51	388
36·3	2.24 „	–	50	167	86	143	61	507
		DAYLIGHT.						
S.	9.19 a.m.	–	36	45	15	21	–	117
4	8.59 „	–	45	121	135	35	11	347
10	8.38 „	26	51	128	375	170	102	852
21·8	8.17 „	–	43	114	499	627	143	1426
25·7	7.52 „	24	121	437	680	850	316	2428
34·3	7.29 „	21	63	169	486	824	549	2112

TABLE VIII.

TOTAL NUMBERS OF SAGITTA SETOSA AT 2-MILLIMETRE INTERVALS OCCURRING AT EACH DEPTH ON JUNE 3RD–4TH, 1926, IN 10-MINUTE HAULS WITH THE 2-METRE RING-TRAWL.

Depth in metres.	Time.	Length in millimetres. <8	8–9½	10–11½	12–13½	14–15½	>15½	Total.
			DAYLIGHT.					
S.	4.1 p.m.	–	2	3	2	4	2	13
4·3	3.44 „	–	15	19	3	12	–	49
10·8	3.25 „	21	21	30	10	10	–	92
18	3.2 „	11	11	30	31	–	–	83
26·6	2.41 „	10	22	52	63	10	10	167
31·5	2.20 „	–	13	68	13	13	13	120
			DUSK.					
S.	9.3 p.m.	14	224	264	149	13	13	677
4·4	8.46 „	8	81	130	61	8	–	288
9·3	8.28 „	15	89	224	275	127	15	745
19·7	8.9 „	–	25	88	50	76	13	252
25·3	7.48 „	–	56	111	144	121	32	464
30·3	7.24 „	–	110	66	171	171	33	550
			DARK.					
S.	12.21 a.m.	–	23	78	78	54	54	287
4·6	12.1 „	18	18	36	77	303	–	452
12·3	11.36 p.m.	–	54	87	45	152	74	412
17·2	11.13 „	–	40	32	40	120	32	264
25·9	10.51 ,	-	22	44	92	102	57	317
35	10.28		36	36	151	266	91	604
			DAWN.					
S.	4.7 a.m.	–	11	35	47	–	–	93
4	3.49 „	–	32	10	64	10	–	116
9·5	3.29 „	10	41	51	105	90	41	338
20·5	3.8 „	21	21	41	41	52	31	207
24·4	2.47 „	10	43	53	23	33	10	172
36·3	2.24 „	–	14	–	14	44	–	72
			DAYLIGHT.					
S.	9.19 a.m.	7	–	–	–	6	–	13
4	8.59 „	22	148	54	10	10	–	244
10	8.38 „	–	18	–	35	54	–	107
21·8	8.17 „	–	21	64	45	64	–	194
25·7	7.52 „	–	41	–	106	125	–	272
34·3	7.29 „	–	65	161	170	251	161	808

TABLE IX.

TOTAL NUMBERS OF SAGITTA ELEGANS AT 2-MILLIMETRE INTERVALS OCCURRING AT EACH DEPTH ON JULY 16–17TH, 1924, IN 10-MINUTE HAULS WITH THE 2-METRE RING-TRAWL.

Depth in metres.	Time.	Length in millimetres. <8	8–9½	10–11½	12–13½	>13½	Total.
		DAYLIGHT.					
S.	4.34 p.m.	12	21	47	18	–	98
6·6	4.18 „	23	33	84	56	–	196
13·6	4.1 „	54	180	502	422	37	1195
19·5	3.44 „	73	146	644	1857	205	2925
34·5	3.25 „	20	187	507	445	31	1190
		DUSK.					
S.	9.7 p.m.	97	534	983	438	8	2060
7	8.49 „	–	–	–	–	–*	1230
15·1	8.32 „	16	143	508	745	48	1460
17·1	8.15 „	80	211	662	1232	105	2290
32·3	7.56 „	74	225	913	1114	74	2400
		DARK.					
S.	12.9 a.m.	83	281	364	312	–	1040
5·8	11.52 p.m.	64	208	640	672	16	1600
11·2	11.34 „	35	70	528	1092	35	1760
20·7	11.16 „	40	118	376	436	20	990
32·2	10.52 „	173	142	692	535	31	1573
		DAWN.					
S.	4.6 a.m.	39	15	12	–	–	66
1·9	3·51 „	63	85	47	8	–	203
9	3.34 „	40	222	394	334	20	1010
16	3.14 „	69	122	245	54	–	490
30·1	2.52 „	181	105	831	393	–	1510
		DAYLIGHT.					
S.	10 a.m.	99	163	89	4	–	355
5·3	9.43 „	205	251	251	53	–	760
12·5	9.26 „	136	543	1667	1020	34	3400
18·3	9.8 „	500	1211	2924	2495	–	7130
31·1	8.46 „	136	271	866	1410	27	2710

* The tube containing these specimens had dried up.

The Lower Limits of Perception of Electrical Currents by Fish.

By

H. C. Regnart, M.Sc., F.Z.S.

In recent years attention has been given to the influence of electrical currents as fish deflectors or screens, and experiments have been made to determine the magnitude of the currents which are sufficient to paralyse or act as deterrents to fish which may enter the electrical field,* but in such strong fields the perception of the mechanism by which fish respond to electric currents is masked by the paralysis produced. It seemed possible that experiments with very weak currents might give fuller information concerning the mechanism by which fish respond to electrical stimulus than those made in strong fields. There is also the possibility that weak electric currents may occur in nature to which fish respond, but before exploring that field of phenomena it is necessary to observe the behaviour of fish under the influence of very weak fields. McMillan found that the field (volts per inch) required to paralyse young salmon varied with the resistivity of the water ; thus when the latter was 10,000 ohms per inch cube the paralysing voltage was about 1·5, so that a current density of $\frac{1{\cdot}5}{10^4}=0{\cdot}00015$ ampere per square inch was sufficient to cause paralysis, whereas when the resistivity was twelve ohms per inch cube the current required was ·03 ampere. In the former case the conductivity of the fish was greater than that of the water, in the latter case less.

In the experiments to be described to determine the lower limit a preliminary investigation was made by applying a current field to freely-swimming fish. When a fish is subjected to such a stimulus it is desirable to measure the current in terms of its density, e.g. amperes per square centimetre, otherwise there can be no adequate standard of comparison of the results of different experiments.

Some initial tests were made, using goldfish (*Carassius auratus*) maintained in a wooden tank 12 ft. long containing fresh water. Into this were fitted vertically two rustless steel plates measuring 30×32 cm. wide which exactly fitted the cross section. The tank was placed in a

* F. O. McMillan, "Electric Fish Screen," Bulletin of U.S. Bureau of Fisheries in Washington, 1929, Vol. XLIV, 1928, p. 97, with current alternating at 60 periods a second.

separate darkened room free from all forms of vibration and other disturbances. The plates were arranged at a distance of 100 cm. apart, and using these as electrodes the effect of various intensities of direct currents on small fish about 6 cm. long was investigated. The tank was provided with a glass window and the fish were observed from a distance by means of a large periscope. The water was kept at a constant depth of 20 cm. Responses were obtained on completing the circuit, and occasionally again in a weaker form on breaking the circuit, the latter being a response to the removal of the current stimulus. The nature of the responses varied according to the position of the fish. When orientated so that the anterior end was facing the positive or higher potential plate, completing the circuit caused a rapid convulsion and generally an immediate retreat. When, on the other hand, the anterior end of the fish was facing the negative plate, there was often no response or sometimes a slight twitch although the current was the same as before. The field was unidirectional.

When the fish swam transversely across the field responses were slight and infrequent. Repeated application of the current every few seconds caused the responses of the fish to become weaker and finally to cease altogether. These responses were very definite with a total current of 100 milliamperes (and a potential difference of 15 volts across the plates) which corresponds to a current density of 0·00016 amperes per sq. cm.

When the circuit was completed and then broken rapidly, sharper responses were obtained. While the current was passing steadily for a short time, the fish were not affected. Similar, but definite though weaker, responses were observed on completing the circuit carrying a current of 10 milliamperes (and a potential difference across the plates of 1·5 volts) for which the density was 16 microamperes per sq. cm.

There appeared to be no sharply defined lower limit at which the fish became sensitive, and they were shown to respond to still lower current densities than the above by the following experiment. The tank was brightly illuminated and a narrow band of shadow, about 4 inches wide, cast transversely across the middle. The fish at first sheltered in the shaded area, but presently began to emerge and swim into the lighted region ; but on making and breaking several times a current of 3 milliamperes (current density=5 microamperes per sq. cm.), the fish could all be caused to retreat and assemble again in the shaded area, but not to exhibit the characteristic sudden responses.

The next experiments were performed in sea-water on marine fish, and the same plates as before were lowered into a large floor pool in the Dove Marine Laboratory at Cullercoats, at a distance of 8 feet apart. The length of this pool was 17 feet, its width 10 feet, and its depth 1½ feet. Three codling (*Gadus callarias*), each about a foot long, were limited to swim up and down between the plates by means of string netting, so that the

available cross section of movement was the same as the area of the plates. With direct current varying between 0·2 and 0·6 amperes (and 2–4 volts potential difference on the circuit including the adjustable resistance, this giving some measure of the initial shock) definite responses were obtained with a pendulum switch set for 0·5 sec. contact, but much better results followed when the current was made by a quick action switch; the pendulum contacts were probably too light. After the response at the make of the current, the fish were not affected while it flowed continuously. The effect was mainly at the make of the current, but occasionally in a weaker form at the break. This indicated that a stabbing switch set to work at short intervals, e.g. half a second, would be more effective as a fish screen than a steady current. As before, the most marked results were obtained when the fish were so orientated that the anterior end was facing the positive plate, with occasionally a slight twitch when facing the negative plate. As in the case of the fish tested in fresh water, the responses to repeated stimulus grew less vigorous until finally no effect was obtained at the make and break of the current. Stronger currents caused more vigorous responses and more tests could be made before the fish ceased to respond. Continued trials carried out over several days adversely affected the fish and made them sluggish and unsuitable for further experiment until they had been rested for some days.

In order to place the fish under more natural conditions the nets were removed. The fish then swam freely about, and when facing the positive plate gave better responses with these currents (0·2–0·6 amps. at from 2–4 volts). A disadvantage of these experiments was that after a few responses the fish avoided the region between the two plates. All these trials were made under suitable conditions with freedom from ground vibrations and other disturbances, and in a weak light. The responses were recorded with the help of three additional observers and no observations accepted unless agreed to by all. Owing to the fact that in this case when working in the floor pool the current between the plates spread out, the actual current densities were from 15 to 45 microamperes per sq. cm.* Similar experiments were made with alternating currents, using the same fish. Under these circumstances the fish gave a slight response when facing the earth plate, but a more vigorous response was observed when facing the high-tension plate. As before, the best results were obtained at the

* Note by Professor W. M. Thornton:

If the current i is taken as spreading out from small cylindrical electrodes the density midway between them is approximately $i/\pi Dl$, where D is their distance apart and l the depth of the liquid. With $i=0{\cdot}2$ ampere, $D=250$ cm., $l=44$ cm., this is equal to 5·8 microamperes. If the current is taken as spreading from strip elements of two flat plates 30 cm. wide and the effect is integrated over their width, the density midway is 19 microamperes. A more complete treatment, assuming the electrodes cylindrical and relatively large (see Pidduck, "Electricity and Magnetism," §46. See also F. O. McMillan, "Electric Fish Screen," Bulletin of the U.S. Bureau of Fisheries, Vol. XLIV, 1928, Fig. 12, p. 117), gives the estimated values 15 to 45 microamperes per sq. cm.

make and break of the current. With a current of 3 amperes and the plates 6 feet apart, very violent responses were obtained. Trials were made with currents over the range 0·5 to 3 amperes, corresponding to the current densities 0·000038 to 0·00022 ampere per sq. cm., at the lower of which response was just perceptible.

In order to investigate the threshold values in sea-water more definitely the following method was used. Twelve codling, about 8–10 inches long, were maintained in a healthy condition in a tank with a glass side which measured 4 feet 6 inches deep, 4 feet wide, and 4 feet long. A wooden frame covered with string netting which exactly fitted the tank was lowered into the water until it was 18 inches from the bottom, and the fish were kept confined in this space with the electrodes arranged at each end and 3 feet 4 inches apart. Under these circumstances, the fish, while swimming freely, were constrained to move more slowly and the sudden twitch due to an electric impulse could be very clearly distinguished. Tests were made at 6-minute intervals. The responses were very definite at first, but after a time became weaker and the fish finally failed altogether to respond. As the current was increased more responses were obtained before the effect ceased. The fish, after two days during which the tests were carried out, became sluggish and refused to feed, and the tests had to be made at least every other day to give them time to recover from the effects of the repeated stimuli and their confinement in a limited space. Alternating current was used over the range 0·025 to 0·65 ampere, which in this case gave the current density range of 2 to 50 microamperes per sq. cm. In these experiments the fish were tested when facing the positive plate and either in the centre of the field or nearer to it. After a time the fish avoided going near the higher tension plate, and this was proved by reversing the leads on the plates. The fish also would avoid swimming frequently between the plates. As before, three additional observers were employed and only vigorous movements regarded as positive ; doubtful cases were taken as negative. These responses were quite distinct from the slower movements made when the current was not passing. They consisted of two movements, (1) a sudden twitch, (2) an immediate change of direction in swimming. A larger number of consecutive readings could not be obtained, as after carrying on the experiments for several weeks the fish as before became sluggish and refused to feed.

Experiments were next undertaken to ascertain if possible the regions which function as receptors of the stimulus of the current field ; and as a first attempt the lateral canals with their sense organs were considered. As before codling about 15 inches long were used. Three fish were anæsthetized by the addition of some chloroform to the water and cuts made on both sides of each fish, which severed the supra-opthalmic, suborbital, and hyomandibular branches of the facial nerve, the lateralis

nerve, and the ventral ramus of the lateralis nerve. These operations eliminated the organs of the lateral canals (and also the skin of the anterior part of the head, since cutting the supra-opthalmic branch of the facial nerve also involved cutting the corresponding associated branch of the trigeminal nerve). The fish were left for a fortnight to recover, and as they had but a few days before been obtained directly from the sea, the recoveries were very rapid. Under similar conditions to those already described, the fish were still found to respond to stimuli of the order of 15–30 microamperes per sq. cm. although they were less sensitive than in the case of unoperated fish. One of the fish with lateral organs eliminated was then subjected to section of the optic nerve on both sides and again the sensitivity to electrical stimuli was not completely eliminated. In all these operative experiments the fish were killed after experiment and the fish after being hardened in spirit dissected to verify the nerve-cutting work. These experiments made it probable that other areas of the fish such as gills were involved in the perception of these electrical stimuli, but the marked reduction in the strength of the response caused by eliminating the lateral organs made it certain that they played a large part in the response to electrical currents. This is possibly due to the relatively high electrical conductivity of the mucus in the canals, but this point requires further investigation.

As a further proof of the role played by the lateral organs as electrical receptors the following experiments were made which show that these organs may have influence in the perception of the direction of the source of an electrical current in the sea. On the application of an electric field to a normal freely swimming fish, the direction of movement of response was found to depend on the orientation of the fish with respect to the field. Thus, when swimming towards the higher tension plate and inclined to it at a slight angle, the response was generally such that it swam away from the plate in a direction either to left or right which removed it most rapidly from that region.

Two fishes were then operated on by in one cutting the supra-opthalmic branch of the facial nerve on the right side and in the other the corresponding nerve on the left side, and on recovery they behaved like normal fish in their directional movements with respect to the positive plate. Finally, two fish were each subjected to section of all the nerves to the lateral canals on the right side ; these fish were then found to give a strong twitch to the left on stimulation whatever their position in the field might be when facing the higher potential plate. These results were obtained over a range of 300 tests and the response to the left was 100 per cent. Next two fresh fish were taken and all the lateral organs on the left side eliminated in each case. These fish gave a twitch to the right over a corresponding series of tests.

These experiments indicated that the lateral canals with their mucous secretions and the organs associated with them may have considerable importance as receptors for directional purposes, and support the suggestion that the greater development of these organs in certain deep-sea fish is for this purpose.*

It should be emphasized that the above experiments were performed upon fish which were exposed to light and other unnatural environmental conditions and that due to confinement and diet they could not be comparable in vigour and sensitiveness with those in a free state ; but even under such unfavourable conditions the results obtained prove that fish are extremely sensitive to electric currents.

The above results may be summarised as follows :

1. Goldfish had a lower sensitivity threshold at about 5 microamperes per sq. cm. direct current.
2. With Codling in a shallow floor pool the threshold was about 15 microamperes per sq. cm. direct current.
3. In a large wall tank their threshold value was about 2 microamperes per sq. cm. alternating current.
4. The lateral organs are proved to play a most important part in the perception of electric stimulus. Section of the nerves of those on the right-hand side caused the fish to throw to the left, and vice versa.

The early experiments were carried out in an annex of the Electrical Engineering Laboratory at Armstrong College ; the experiments on sea fish were made by kind permission at the Dove Marine Laboratory, at Cullercoats.

I am greatly indebted to Professor W. M. Thornton for suggesting the research and for his constant help and advice.

* Vide "Electrical Perception by Deep Sea Fish," W. M. Thornton. University of Durham Philosophical Society. Proc., Vol. 8, Part 4.

Some Observations on Schooling in Fish.

By

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With 12 Figures in the Text.

CONTENTS.

PAGE

I. Introduction 421
The Schooling habit 421
Problems connected with Schooling 422
Bass as a subject for experiments on Schooling 425

II. Description of Experiments 426
Preliminary 426
Showing that sight is involved—Reactions of the fish to a mirror . . 431
Summary of experiments with a plain mirror 433
A. Reactions of individual fish 433
B. General power of attraction of a mirror as determined by comparison of average positions of the fish 437
The main attributes of that which induces a Schooling response . . 442
Reaction to mirrors used as a basis for further experiments . . . 444
Reaction of fish to small areas of mirror 445
Reactions to a broken mirror surface 445

III. Application of Results to the Schooling Problem 446

Summary 447

I. INTRODUCTION.

The Schooling Habit.

The habit among certain kinds of fish of living in herds, or "schools," is too familiar to require much in the way of introduction. Those fish in which the habit is most strongly developed, such as members of the herring and mackerel families, though scarcely developing such organisation as can be seen in some bird flocks, clearly display a considerable degree of mutual adjustment between individuals. The term "school" or "shoal" is applied to fish herds which are not mere chance aggregates brought together by the common attraction of some external condition (e.g. collections of salmon on migration up river) but definitely involve mutual reaction of one individual to another.

Almost every stage can be found between regular school fish and those in which temporary bunching together may occur on certain occasions, as Parr (**3**) has pointed out, giving *Gobius ruthensparii* as an example of a simple case. "The adults of this species generally do not school at all, but when splashing among the algæ where they are scattered one may very commonly observe that they do not dart off in all directions, but converge to form a school of very short duration, spreading again after a short flight."* Other kinds form more stable, though not permanent, schools. As an example there is the European Stickleback (*Gasterosteus aculeatus*), "which will travel in rather perfectly formed schools but will scatter for breeding and feeding. Similar behaviour also seems to be found among the American killies (*Fundulus*) and many other fishes" (p. 29). The bass (*Morone labrax*), in its immature stages, lives in considerable schools, but as it grows older becomes increasingly less sociable. During the shoaling period, however, the habit is not so dominant as to preclude considerable independent individual action, and has its main effect in keeping the group together, rather than in co-ordinating movements.

From such cases we pass to permanent school fish, such as members of the herring and mackerel families, in which the school is extremely stable and is not broken up unless there is violent disturbance. Since these fish are continually on the move, for feeding as well as travelling, their case differs from that of *Gasterosteus aculeatus*, etc., only in degree. The behaviour of individuals of these fish leaves an impression of being extremely stereotyped, and non-variable, and specialised in the development of actions suitable for the maintainance of the school. That these actions are developed to a degree of some refinement is apparent from the regularity and co-ordination of movement so characteristic of the schools. So, when the individual is considered, it may well be asked what are the nature of its actions that result in the school apparently working as a unity. Here is a subject for enquiry.

Problems Connected with Schooling.

The phenomenon of schooling has received surprisingly little attention either from fishery investigators or from those studying animal behaviour. Parr (**3**), it would appear, has been the first to examine the subject in the light of cold reason, and to apply a form of scientific treatment to it. But the subject is here approached from a somewhat different aspect.

Setting ecological questions aside for the time being, and keeping an

* The habit of packing together when scared is common among true schooling fish, as has been observed in the case of mackerel kept in the aquarium, and is well known to occur when sprat, pilchard, etc., are attacked by sea birds. It is possible also that it occurs among non-schooling fish such as hake (Bateson **1**, p. 249). If so this is a case of schooling in its most rudimentary form.

analytical standpoint, the problems connected with schooling, whether permanent or temporary, well developed or indefinite, may be expressed as follows :—

1. Given the phenomenon of schooling.

(*a*) What sort of actions on the part of the individual are concerned in keeping the school together, and how can various activities displayed by the school be interpreted in terms of behaviour of single fish ?

(*b*) What are the essential elements in the aspect of its surroundings to which the individual reacts, and which in general influence its behaviour ?

(*c*) From the special aspect of sense physiology, what are the senses concerned ? How refined are these senses ?

2. Given a fish liable to display schooling behaviour.

(*a*) Under what conditions will that behaviour be displayed ?

(*b*) How determinate is that behaviour—to what degree of probability can it be predicted ?

(*c*) To what degree of certainty can it be experimentally induced ?

(1) Questions 1 (*a*) and (*b*) cannot strictly be separated from each other, being really only two ways of propounding the same problem. The one focuses attention on the animal's actions, the other on the surrounding conditions. But actions cannot be described without some sort of reference to environment, and the effect of the latter can only be stated in terms of actions being performed at the moment. Only in limiting cases does a clear distinction emerge ; the first enquiry dealing with "reactions," the second with "stimuli" producing them. And as for experimental purposes it is generally convenient to deal with clear-cut reactions, these two aspects of the phenomenon may be kept separate.

(1*a*) This is largely a matter of description, but observation should be made on whether the individual behaviour is variable or rigid, complicated or consisting of a limited set of more or less cut and dried reactions. Naturally, the more stable and mechanical the movement of the school, the more automatic the actions of the individual fish are likely to be. It is of interest to know how strongly developed and how permanent are the reactions involved ; e.g. will a fish always react to a neighbour in a similar way whether forming part of a school or not ? It is further to be noted how far external conditions influence the observed behaviour. For instance, when a school is feeding there are not such rigid adjustments between individuals as when it is travelling ordinarily ; in the former case like orientation of all the members of the school may completely disappear.

(1*b*) Here one is particularly concerned with such questions as "How do the fish recognise each other ?" Or rather, "What distinctions do the

fish draw between neighbouring fish and other objects ? " Or, put another way, " What are the essential elements in a situation which results in a schooling response ? " In any case, however the question is framed, it is not intended that it should convey anything beyond an objective meaning. Experimentally a fish, which reacts in a definite way when another fish comes near it, is tested under various circumstances in which some factor in the normal situation is lacking. The features shared in common by those situations which produce the normal reaction may be considered as their essential elements. By this means evidence may be procured as to the importance of movement, form, general contrast, details, spatial relations, and so on. The extent of the data obtainable depends on the strength and persistency of the original reaction.

(1*c*) By treatment of the animal as a unit, it is possible to demonstrate empirically the dominant sense involved ; and to deal with such questions as the following : " Assuming sight to be involved, at what distance (for given illumination and turbidity of water) will a fish exert attraction on another ? " " How complete an image is necessary and what are the essential elements of it ? " " What illumination is necessary ? " Further, the degree of refinement of the sense may be tested with considerable accuracy by conditioning methods. But neither these nor other exact analytical methods of sense physiology are here utilised, and so further consideration of them is unnecessary.

(2) Once observation is undertaken on fish in the above connections, this second side of the problem is bound to open up. The answers given to the questions concerned can at best be approximations, and in any case only apply to specified laboratory conditions. As for elucidation of behaviour under natural circumstances, they cannot legitimately be used except as evidence (often, however, this evidence, combined with that of what observation is possible in the field, is sufficient to give an adequate answer).

(2*a*) By this is implied not only what are the conditions under which fish maintain a school, but also what changes are necessary to convert a " non-schooling " state of the fish into a " schooling " state, and vice versa.

(2*b* and *c*) To deal with these questions fully would involve the whole subject of determinancy with respect to behaviour actions of animals. It is enough to record such observations as bear on the points. Empirical treatment is given to these questions not merely for the sake of cautious procedure, but because theoretical consideration leads round to this as the only permissible method. The questions are framed in a way which does not presuppose tropistic behaviour or indeed any causal relations whatever, but if actions exist that are sufficiently fixed to be conveniently

termed " tropisms," they will appear during investigation. The habit of postulating hypothetical tropisms constant only in hypothetical ideal conditions (so that in practice they would be partially obscured by secondary influences) is considered a fallacious method of treatment.

In this paper an account is given of certain observations on the bass (*Morone labrax* L.). These are discussed in their bearing on the problems outlined above in so far as they concern that species.

Bass as a Subject for Experiments on Schooling.

Choice of schooling fish suitable for observation in captivity is limited to a very few species. Small mackerel, herring, and sprat are excluded on account of difficulties involved in keeping them in confinement. The species most readily obtainable was the bass (*Morone labrax*), a fish which, though not such a specialist as the herring or mackerel, has quite a strong tendency to form schools.

The bass is a lively, vigorous fish with a traditional reputation for capriciousness and wariness. Admittedly, when all the facts known about its habits are considered, one cannot avoid the conclusion that its behaviour is normally far less stereotyped than that of most fish, showing much diversity and adaptability. Its diet, which is principally animal, preferably alive, covers a wide range from seaweeds to sewage effluents, and various are the methods adopted by the fish in obtaining it. The bass also shows considerable powers of resistance to environmental fluctuations such as changes in salinity and lack of oxygen. It lives well in captivity, surviving conditions that would be detrimental to most fish. In captivity it is seen to be alert and quick to take alarm—in fact, gives the appearance of living in a considerable state of nervous suspense—but is capable of becoming habituated to such conditions, if constantly repeated or continually present, as at first produced fright reactions.

The schooling habit is best developed in the younger fish which invade estuaries during summer months. At this period, especially in the earlier part of the season, while feeding vigorously, they form considerable schools, and even when the larger schools disperse the fish still keep together, at least in small parties. Schooling behaviour appears to weaken as the season advances. It has been observed (**2**, p. 117) that fish of the same size school together.

Summarily compared with other schooling fish, the bass may be said to represent a type in which schooling behaviour is not developed to such a predominating extent that it is practically stereotyped into automatic action, but rather, so to speak, exists as one of several forms of behaviour which at different times are liable to emerge from the general " behaviour pattern." In other words, it is a good generalised type in which schooling

behaviour is present, but not in an exaggerated form. Compared with the schooling habit of specialists like the herring, that of the bass is not so well defined, nor so deeply engrained, nor so persistent. A variable element in seasonal change has been indicated above. Observations in captivity show that there is a considerable difference in the intensity of this behaviour under different circumstances, being stronger, for instance, when the fish are alarmed, and weaker when hungry. By the comparison of the behaviour of bass kept in an aquarium tank with that of grey mullet and mackerel under similar conditions and with sprat in tidal pools, it was seen that of these four species the orientation of individuals in relation to one another is least evident in the bass. In this species also there is much scope for independent individual action. In fact, when a number are together in a moderate sized tank, there is little, if any, evidence of schooling behaviour. Yet, as is shown below, individual fish have a strong attraction for each other.

It is this attraction between individuals that was made a basis for the experiments here described.

II. DESCRIPTION OF EXPERIMENTS.

Preliminary.

Preliminary investigations were started in October, 1929, on four fish, between 6 and 7 inches long, taken from the tank in the aquarium where a considerable number had been living for some months. They were placed in a tank in the main laboratory. A view of the interior of the tank ($58\frac{1}{2}'' \times 31''$ and $18\frac{1}{2}''$ deep, holding a depth of water of $14''$) was obtainable through the front which, except for the lower 6 in., consists of glass. The other walls of the tank are dark and the glass was covered with black paper, leaving only a space for a peephole in the middle. When not in use the peephole was kept covered over. Besides this, no attempt was made to exclude such disturbing influences of the surroundings as might be reflected in the behaviour of the fish.

The difference between the surroundings in the aquarium and those in the laboratory tank were considerable and it is not surprising that for some time the behaviour of the fish was dominated by "fear" or "response to disturbance" actions. At any rate, they were much inclined to keep to the far side of the tank, lying low in corners or behind a vertical outlet pipe in the centre. This, on the one hand, contrasts with the behaviour in the aquarium tank, in which the fish displayed considerable activity, swimming high in the water and attracted rather than repelled by movement outside the glass; and on the other hand is clearly related to behaviour towards a disturbance, from the side or above, when the fish, having at first darted rapidly to and fro, take up a position of rest low

down in a corner or under some shelter if available, and lie quiet facing the source of disturbance.

As time went on the fish became less and less inclined to lie low at the back of the tank. Movement at the peephole had increasingly less effect. In fact, when the observer was quite still and took care that as little light as possible entered through the peephole, the fish behaved unconcernedly, so long as they did not swim up too close.

During observation on these four fish in daylight, with the aid of glass partitions set up in the tank, the following conclusions were soon reached.

(1) Fish placed in a tank, especially if disturbed, collect together and are restless when separated.

When their "attention was attracted" they bunched together orientated towards the source of disturbance. If one began to move away the others followed. But they also kept together when undisturbed. All through subsequent experiments when more than one fish was used the tendency was very evident.

(2) Fish separated by a glass partition move towards each other and school on either side of the glass.

The following tests were made :—

(*a*) Two glass plates (XY, YZ) were placed in the tank as indicated in Figure 1. The usual position of the fish (when the tank is clear) is along AB. When the plates were put in, one fish was separated from the other three which were penned in the area XYZ. When observation began the three inside fish were lying behind the vertical outlet pipe (OP), while the single fish lay up against the glass at X. The three fish moved in the direction of B ; whereupon the single fish made great efforts to follow them, persistently swimming up against the glass XY.

This was repeated on two other occasions with the same result.

The outside fish was observed attempting to pass XY when the other fish had come to rest near Z. The attraction of the one for three was always greater than that of the three for one.

A black plate, PQ (Fig. 2), was placed against XY so as to cover it for much of its length, leaving, however, QY free. Whereas (1) before this the fish had attempted to pass through region XQ, (2) when the plate was put in it kept to region QY ; and (3) when the plate was shifted to P^1Q^1 it attempted to pass XP^1.

(*b*) Three fish were penned by glass plates within area XZ (Fig. 3), one left outside.

Outside fish, previously, when entirely alone in the tank, was not observed to come forward at all from region AB.

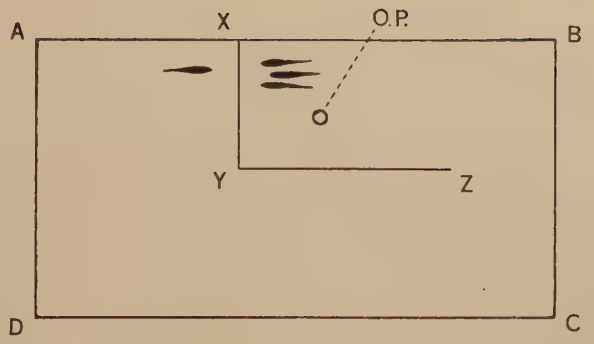

FIG. 1.

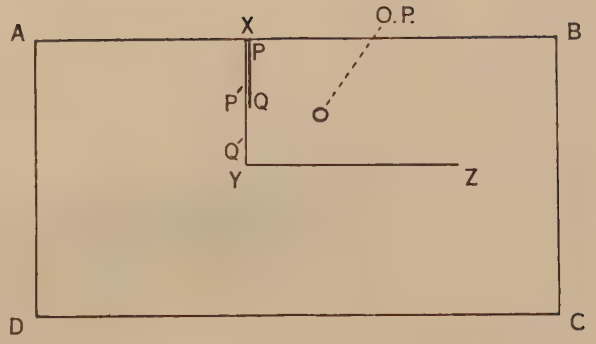

FIG. 2.

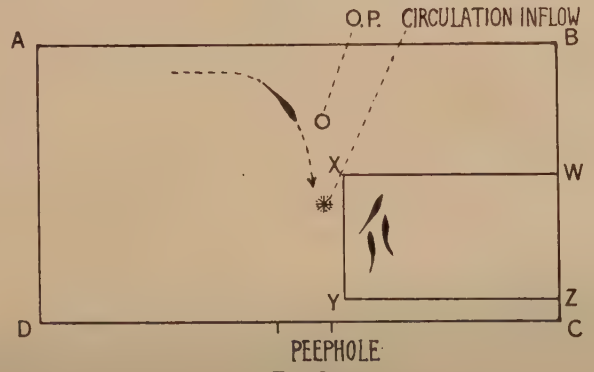

FIG. 3.

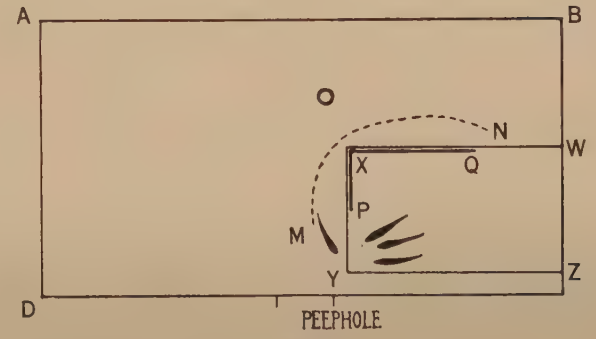

FIG. 4.

FIGS. 1–4.—Diagrams of the arrangement within the tanks on particular occasions during the course of the experiments. (See text.)

ABCD= Outlines of tank. W, X, Y, Z in all cases represent glass partitions standing vertically in the water. They were supported at their lower corners by bricks, which are not shown in the figures. O.P.= The overflow pipe standing vertically in the water.

But when the three fish are in XZ the outside fish was seen on many occasions to swim forward towards the peephole along XY.

Note.—The outside fish came up more readily when the circulation inflow was striking the water at the point marked with a star, close to region of XY, and breaking the surface. On one occasion the observer was looking down from on top. When the circulation inflow was turned off or diverted into XZ, so clearing the view, the fish did not come forward when the observer was looking down into the tank, but did so when the observer was keeping still at the peephole.

The outside fish now and again tried to pass into XZ between X and Y, and in one case between X and W.

Black plates were put in positions XP and XQ (Fig. 4). (i) The four fish took up position on either side of PY, thus indicating that the inside fish were affected by the other and not merely the other way round. (ii) Subsequently the inside fish took up various positions; the outside fish spent much time moving round arc MN, now and again attempting to join the inside fish either at QW or PY.

The black plates were tried in other positions, always with the same results; the outside fish attempted to pass through the clear glass spaces towards the inside fish.

All results were seen to be independent of the position of the circulation inflow—this did not appear to offer any attraction. It could be used with advantage as a screen.

(*c*) The tank was divided down the centre by a glass partition (Fig. 5).

Normal position of fish somewhere along AB.

Two pairs of fish are separated by the glass.

If left alone they collect on either side of XY and orientate themselves, as a rule, in the same direction. They frequently are observed attempting to pass through the glass partition, and will snap at food falling on the other side of the glass.

If a black plate is placed at XY the fish take up a position at P. If frightened back they come forward again in a short time, even though they may be moving towards a source of disturbance.

XY glass only . . .	Fish greater part of time at XY.
XY glass+black plate .	Fish for a larger part at P.

However, two pairs of fish do not exert the same mutual attraction as two single fish or as one fish towards three.

The mutual attraction that exists in these fish is best seen, then, in the case of a single fish separated off from a batch of others.

(3) The attraction of one fish for another may be strong enough to overcome what would otherwise be repelling effects.

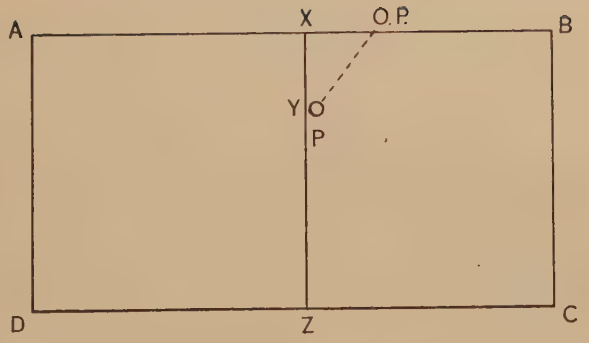

FIG. 5.

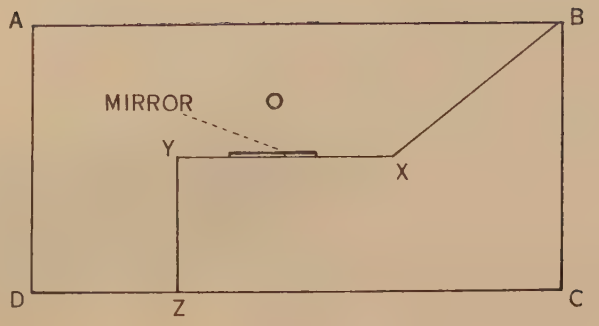

FIG. 6.

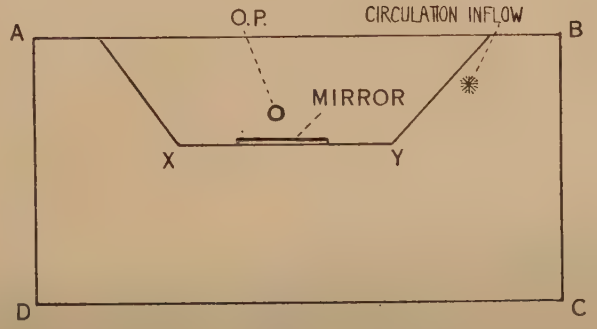

FIG. 7.

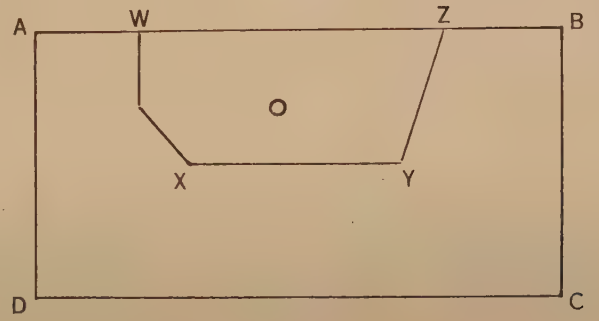

FIG. 8.

FIGS. 5–8.—For description, see Fig. 1, pages 427, 428.

An instance of this has been given above. An isolated fish moves towards peephole when attracted by fish penned in glass.

In addition there was good indication that :—

(1) The fish do not readily, if at all, "sense" the glass plates. They will swim into them, and often persist for some time in swimming against them when held up.

(2) The fish behave as if reacting to each other by *sight*, the evidence being :—

(*a*) A fish separated from others by glass alone will make conspicuous efforts to pass through towards other fish. But this does not happen if they are separated by an opaque screen, although enough space is left for the passage of dissolved substances or vibrations in the water.

(*b*) There is never any reaction to the spaces or crevices on the edge of the separating plates.

(*c*) When a fish is attempting to pass through a partition which is partly opaque and partly transparent, it will only attempt to pass through the transparent parts.

Showing that Sight is Involved—Reactions of the Fish to a Mirror.

In order to test out the proposition that bass react to each other by means of sight, experiments were carried out on the behaviour of the fish in the presence of a mirror.

From this point a second tank (59″ × 30″, holding a depth of 15″ of water) was used, situated in a part of the laboratory where the light was dimmer and in general less liable to disturbing influences. In order that the fish might be observed without being disturbed at all, a hood of black cloth was hung over the side of the tank (in addition to black paper stuck on the glass), arranged over the peephole in such a way that all light from behind was cut off, and the observer's face rendered invisible.

The arrangement proved entirely satisfactory, its effectiveness being finally estimated by the influence, if any, on the behaviour of the fish. The fish frequently swam alongside the peephole showing not the least concern for any strange appearance that may have intruded upon their visual field. It was possible, however, if right up close to the glass, to produce by movement a reaction on the part of the fish.* It is beside the point to consider whether the fish detected the observer (whatever that means) or not ; what matters is whether behaviour was affected or unaffected. And as in this case there was a clear contrast between

* For kind of movement, see below.

"behaviour affected by movement at peephole" and "behaviour unaffected," it was possible to estimate when the latter condition held.

The reactions of the fish when coming in front of a mirror are best seen in single specimens isolated from the rest. If in a moderately active state, it spends a good deal of time swimming to and fro across the mirror surface with its face close against the glass as though attempting to pass through it. It passes up and down, often making futile snaps at the surface and displaying other reactions characteristic of frustrated effort. When the head passed beyond the field of the mirror, the fish very frequently pulled up short and turned back. This action, very striking on occasions, showed clearly that the abrupt change from a visual field containing the reflection to a visual field without it may provide a sufficient stimulus to produce a sharp reaction in the fish. Since precautions were taken to have the background approximately similar in the two fields, the difference between the two fields is reduced to that between presence and absence of image, and it is safe to infer that the sudden disappearance of the mirror image in the visual field of the fish is enough to stimulate a sudden reaction.

When the fish is in a less active state it lies up against the mirror for hours at a time. If driven off it will be back again before long.

Particular cases are described below. Small specimens of 3 and 4 inches reacted in a most energetic manner.

The behaviour thus outlined suggests that the mirror image exerts an influence identical with that of the presence of another individual, evoking a schooling response. Definite proof, however, that the image itself really counts for something was furnished during the course of various experiments. One particular instance is of itself practically conclusive.

Glass plates were arranged as shown in Figure 6 (BXYZ). There is a single fish in the tank in front of the glass (area YC). Along XY is placed a mirror covered with black paper except for a rectangular patch of $1 \times 2\frac{1}{2}''$. During a period of two days the fish is observed to react to its reflection in the mirror exposure and to lie up against it for hours. After this a piece of plain glass similarly covered with black paper with a similar $1 \times 2\frac{1}{2}''$ opening was substituted for the mirror. The fish did not react to the opening in at all the same way, but still rested in the region in front of it. The mirror was then replaced, this time along YZ. The fish just afterwards reacted to its reflection and remained in the region before YZ. Mirror and glass were then interchanged and the fish returned to its former position. The interchange of mirror and glass was repeated several times and in every case the distinction in the behaviour of the fish in front of the openings was most definite, the mirror being invariably "discovered." The effects of relative position being excluded by interchange of the plates, the only difference between the two rectangular openings in the black surface was that one contained a reflection of the fish and the other not.

As might be expected, the mirror exerts less attraction on two or more fish together than on one isolated individual—progressively less as the number increases. Nevertheless the same sort of behaviour is seen and individual fish may for a time react quite intensely.

Summary of Experiments with a Plain Mirror.*

A. *Reactions of individual fish.*

1.

Eleven days after the original four fish had been placed in the first tank, they were presented with a rectangular mirror in a brown wooden frame. Being evidently "put off" by the unfamiliar appearance of the frame, none of them behaved in a way which gave much evidence of the effect of their mirror reflection. But when one of the fish (A) was moved to the second tank, the mirror being stood across one corner, a most definite reaction was seen. Since the whole situation was new for the fish, it might have been expected that the mirror and its frame would not exert the same repellent effect as when placed among already familiar surroundings. Again, whereas the fish had always been accustomed to the presence of others its own kind and size, it was now alone. Further, since it had been subjected to the shock of capture in a net and transference from the tank to another, its shoaling instinct should be strong.

It was found that in whatever part of the tank the mirror was placed, wherever the fish was driven, it always returned to the mirror after a short time. Now it swam over the surface, obliquely, with its head against the glass, as though trying to pass through to the other side of the frame; now around in the region close in front of the glass. When it passed out of the sphere of the mirror, it always turned back again, and so, even when most active, remained in one very restricted area. The turning back was most striking when the fish moved along the mirror's surface. On passing the frame (as it was constantly doing) it immediately turned back sharply and often moved in an agitated way until its reflection appeared before it once more.

When not active the fish lay alongside the mirror.

The mirror was placed in different parts of the tank; in every case the fish was attracted to it, behaving in the way described above.

This sort of behaviour has been found to be typical, though it may not always be so intense or so definite.

* These were carried out mostly in daylight, but sometimes in the evening under artificial light. The fish behaved in the same way in either case.

2.

Another fish was then placed in the tank with the above. The hood had now been fixed over the tank so that the fish could be observed without being distracted by the observer. On the first day the reaction was weak. On the second day it was quite strong : every time the fish were observed they were in the region of the mirror, either resting by it or moving slowly round in front of it. On passing beyond the view of their reflection they turned sharply back.

The next day the mirror was removed from its frame and used from now on as a simple rectangular plate ($10'' \times 8''$). The fish were immediately attracted to it and for the rest of the day their reaction was quite strong.

The mirror was then placed in the position shown in Figure 7, the region behind the vertical outlet pipe being cut off by glass partitions with mirror lying up against the longitudinal partition XY facing the peephole. The field of the mirror differed from that of the adjoining glass in that the reflected wall stood further back than the real back wall seen through the glass.

On the first two days the fish, whether active or not, showed quite a strong reaction to the mirror. When active they swum around in the region between mirror and peephole, constantly turning abruptly towards the mirror when the reflections disappeared from the field of view ; when inactive, they lay up against it. On one occasion one of the fish got inside the partition ; it was much attracted by the outside fish which was busy reacting, in turn, to its own reflection.

Two special cases which showed the effect of the mirror image may be mentioned. On one occasion a fish moving along the line from C to D, on arriving opposite the mirror (about 14 in. away) turned at right angles so as to approach it. Another time one fish was lying against, and at an angle to, the mirror ; the reflection of the other, moving in the opposite direction, came into view ; whereupon the former turned and orientated itself accordingly.

Some tests with different models were then made, after which the mirror was again used. It was placed an inch or two behind the glass partition. This was eight days after the fish had been put in the tank. The reaction to the mirror was now distinctly weak. The fish were at first moving about actively and paying a certain amount of attention to the mirror ; but not greatly attracted to it. Occasionally they try to pass through the glass towards it. But they mostly lay quiet in the left-hand side of the tank, and returned there even if shifted toward the mirror.

The fish seemed to be developing a " depressed condition," so, as a test, the mirror was removed and another fish placed inside the glass enclosure. The original fish were not in the least attracted—hence the failure to react

to the mirror was accompanied by failure to react to other fish. The mirror was replaced, but the fish gave no reaction. They were then removed.

3.

Two new fish, netted in the Tamar Estuary a week before, were tried with the mirror arranged in a similar manner as above, but now the glass partition was further forward, half-way between the front and back walls of the tank. This gave less contrast between the background of the mirror and that of the plain glass. (Fig. 8, mirror against centre of XY.)

During the two days these two fish were watched they were greatly attracted by the mirror, spending much time swimming slowly between it and the peephole and not straying from this area. They do not try to pass through the mirror, nor are much inclined to lie beside it ; but then they do not closely attract one another. The reaction of these two fish is good, being much like that of the fish mentioned above.

4.

The behaviour of two small fish (K and L) of 4 in. and one (O) of 3 in. was observed on several occasions. At this size much greater activity is shown than by larger fish. The reaction to the mirror, though variable, was typically most vigorous.

On the first occasion on which the small 3-in. fish (O) was observed in front of the mirror it was alone. The mirror was put in the tank at 3 p.m. At 3.15 the fish was busily swimming before the mirror, in a state of great activity, darting to and fro in front of it and continually swimming at it as though to pass through it. On these latter occasions the dorsal fin was frequently raised, an action which is characteristic of impeded movement. Every few minutes the fish was observed and all the time, until the last observation at 5.50, it was still reacting to the mirror in an astonishingly persistent manner. The next day the tank was divided down the centre by a glass partition and a single 6-in. fish placed on the right, the small fish and the mirror being on the left. In spite of such distraction as may have been provided by the larger fish, the small fish still continued reacting strongly to the mirror, and if it moved away very soon returned.

Each of the 4-in. fish, when tested separately, behaved in a similar way, remaining for a long period in front of the mirror. At times they lay still against the mirror, but for the most part they swam actively up and down and to and fro over the surface, frequently raising their dorsal fin in agitated effort to pass through, and returning whenever they happened to move out of the range of the mirror reflection.

Fishes K and O together reacted strongly in the same way.

Four days later the three fish were put together in a clear tank. The

mirror was stood up against the vertical outlet pipe for half an hour, but during this period the fish paid little attention to it. They were not attracting each other to any extent and passing the edge of the mirror was not sufficient stimulation to make them turn back to their reflections. It was noticeable that, on swimming past the mirror, they almost invariably wheeled and swam round behind the outlet pipe. Again, when, immediately after, the mirror was stood across the space behind the outlet pipe facing the side walls, the fish spent little time in front of it.

In contrast to this, three days afterwards it was observed that the three fish together reacted readily to the mirror. At first the tank was clear ; it was then partly divided down the middle by a glass partition as in Figure 10, the mirror being placed against the right side wall. At first there was only one fish in the right half, but very soon afterwards all three were on the right in front of the mirror.

These last examples provide an illustration of how behaviour is apt to vary from one occasion to another.

As a rule, however, it may be said that the small 3- and 4-in. fish react in a very decided fashion.

5.

Many further instances were obtained showing the attraction exerted by the mirror on individual fish. The behaviour exhibited by them was always essentially of the kind described above.

Tests were also made on a group of about twenty fish and the reactions of individuals were seen to be of the same definite character.

6.

In connection with the appreciation shown by the fish for their reflection, their behaviour with respect to reflecting surfaces lying on the floor of the tank has some bearing.

To a *plain mirror* reflecting light upwards the fish react in a most distinct and peculiar way. They swim over the glass, with the axis of the body tilted upwards (sometimes practically upright !) and snout close against the surface, in a rapid zigzag motion. They keep within the confines of the area of the mirror, turning inwards on reaching the edge.

To a *piece of dark glass*, lying on the floor of the tank or up against a sloping face of glass, they react in a like manner, but not so intensely. A distinct reflection is appreciable to the human eye.

To a *piece of plain glass* lying on the floor of the tank, a similar reaction is given, but it is far less decisive. There is still some sort of reflection in this case.

To a piece of plain glass tilted so that one end is raised and the other

rests on the floor of the tank, there is no reaction. Reflection appears to be absent in this case.

To a piece of silver paper (which had previously been crumpled) lying under a piece of glass on the floor of the tank, there is even less reaction —when indeed there is any—than to plain glass alone.

The evidence of these facts indicates strongly that the mirror reflection is the essential element in the situation which evokes this characteristic behaviour. It is not merely the smoothness of the surface—a tilted piece of plain glass does not produce any reaction at all. The effect of dark glass shows that brightness is not essential, and of silver paper that brightness alone is ineffectual. Smoothness and brightness being thus eliminated, it must be concluded that the actual reflection of the fish, or some general characteristic of it, is concerned. The positive evidence for this is that the intensity of the fish's reaction decreases with decrease in clearness of the reflection.

7.

These, then, are examples of cases in which bass were observed to behave in a distinct manner when in the presence of a mirror. This behaviour is comparable in kind and intensity to that towards other fish behind a glass partition. This serves to show—even in the absence of other evidence—that in this species vision plays a leading rôle in schooling. The reflection calls out the same reactions as would real fish under similar circumstances. It "stimulates schooling reactions"; it creates a "schooling situation."

As far as the actions of individuals are concerned, they are nevertheless apt to vary in intensity from one occasion to another and are far from automatic. They vary even with the fish's activity. Indeed, on the whole, though the type of behaviour is characteristic enough, there is no definite cut-and-dried reaction which could be used as a basis for detailed experimentation.

B. *General power of attraction of a mirror as determined by comparison of average positions of the fish.*

1. Single fish.

For this test a fish was specially chosen which was hardly appreciably reacting to its reflection and which, to all appearances, was scarcely attracted to the mirror at all. An attempt was made to compare the frequency with which the fish was positioned in front of the mirror with that in front of a plain glass with similar background. The fish never lay for any length of time in front of the mirror; could it be shown that it was more frequently in front of the mirror than in front of a similar patch of plain glass?

The glass plates were arranged as in Figure 8. The front 28-in. plate was covered with black paper (kept in place with elastic bands) leaving two square openings, each 14×13 cm. A mirror is placed behind one of these and the other space left clear. The reflected background is very similar to the actual background seen through the clear space.

The frequencies of the occurrence of the fish in front of the two spaces are compared with one another. By this means an indication may be given as to whether the presence of the mirror makes any difference.

The fish is rather sluggishly disposed and shows little definite reaction to the mirror. Hence if any results are obtained from it, other fish should give more decisive results.

During the period this fish was being observed, a note was made every time it was seen in front of one of the spaces (irrespective of the reaction to it) or in the region between, and this was indicated in the accompanying table by a +. The time is divided up into ¼-hour periods, in each of which no more than one + is available for any one column.

The results obtained were as follows :—

		Plain glass.	Region between.	Mirror.
6.viii.30	9–9.15 a.m.	–	–	+
	9.15–9.30	–	+	+
	9.30–9.45	–	+	–
	9.45–10.0	–	–	–
	10.20	–	–	–
	10.45	–	–	–
	12.0–12.15 p.m.	–	+	+
	12.30–12.45	–	–	+
	12.45–1	–	–	–
	2.45–3	–	–	–
	5.45–6	–	–	+
	6–6.15	–	–	–
7.viii.30	9.45–10.0	–	–	+
	11.15–11.30	–	–	+
	12.0–12.15	–	–	–
	3.0	–	–	–
	(Mirror changed over)			
	3.45–4.0	+	–	+
	4.0–4.15	–	+	+
	4.15–4.30	–	–	–
	4.34	–	–	–
	5.10	–	–	–
8.viii.30	8.45–9.0	–	+	+
	9.15–9.30	–	–	+
	9.30–9.45	–	+	+
	10.15–10.30	–	+	+
	10.45–11.0	–	+	+

		Plain glass.	Region between.	Mirror.
	11.0–11.15	+	+	+
	11.15–11.30	–	+	–
	11.30–11.45	+	–	–
	11.45–12.0	+	+	–
	12.0–12.15	–	+	–
	12.15–12.30	–	+	–
	12.30–12.45	–	+	–
	1.15–1.30	–	+	–
	8.30 p.m.	–	+	–
	8.45–9.0	–	+	+
	9.0–9.15	–	–	–
11.viii.30	9.30–9.45 a.m.	–	–	+
	11.15–11.30	–	–	–
	11.45–12.0	–	+	+
	12.45–1.0	–	–	–
	2.30–2.45	–	–	+
	3.0–3.15	–	–	+
	3.30–3.45	–	–	+
	(Mirror changed over)			
	4.15–4.30	–	+	+
	5.0–5.15	–	–	+
	5.15–5.30	+	–	–
	5.30–5.45	–	–	+
	8.30–8.45 p.m.	–	–	+
	9.0–9.15	–	–	+
	9.15–9.30	–	–	+
	Total	5	19	27

The figures representing Mirror : Space between : Plain glass are 27 : 19 : 5, showing a very decided balance in favour of "Mirror"—there is a greater probability that the fish will be in front of the mirror than in front of the plain glass.

2. Aggregate of fish.

With a number of fish in the tank an attempt was made to show that the presence of a mirror would affect their distribution, i.e. if the mirror were placed at one end there should be, on the average, more fish in that half of the tank than in the other.

For this purpose the tank was divided down the middle with a glass plate, leaving a gap just in front of the peephole (Fig. 10). The tank was that used in preliminary tests, with no hood or any special precautions for rendering the observer invisible.

There were 21 fish of between 6 and $7\frac{1}{2}$ in. in the tank. They moved about the tank on the whole quite actively, and when left to themselves soon equalised their numbers in the two halves. At intervals the numbers in the two sides were counted, observation being made through the

peephole. Owing to the observer's presence, the fish would no longer swim to and fro in the connecting space ; so the numbers in either part of the tank could be counted at leisure. For the most part it was sufficient to count the fish in that part of the tank in which there were least.

The distribution of this group of fish in the two halves of the tank is represented graphically. Figure 11 (*a* and *b*) shows normal condition, the

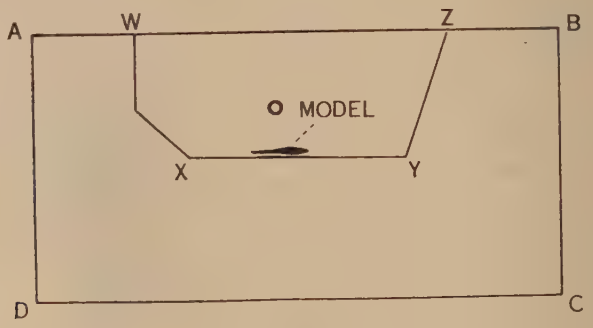

Fig. 9.

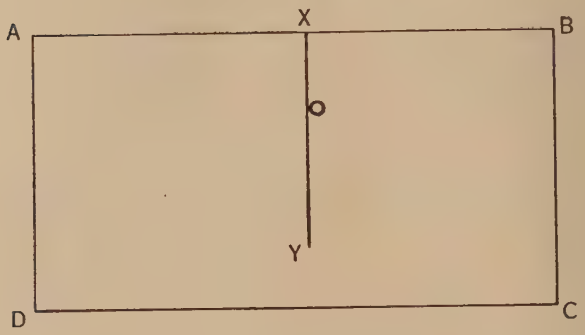

Fig. 10.

Figs. 9–10.—For description, see Fig. 1, pages 427, 428.

fish being distributed with equal probability of preponderance in either half. Figure 12 (*a* and *b*) shows the effect of a mirror standing up against one of the side walls.

There is a clear distinction between the two cases. The results show : (*a*) the probability of a larger proportion of fish in that half of the tank which contains the mirror ; (*b*) the marked change in distribution following the change of the mirror from one side to the other.

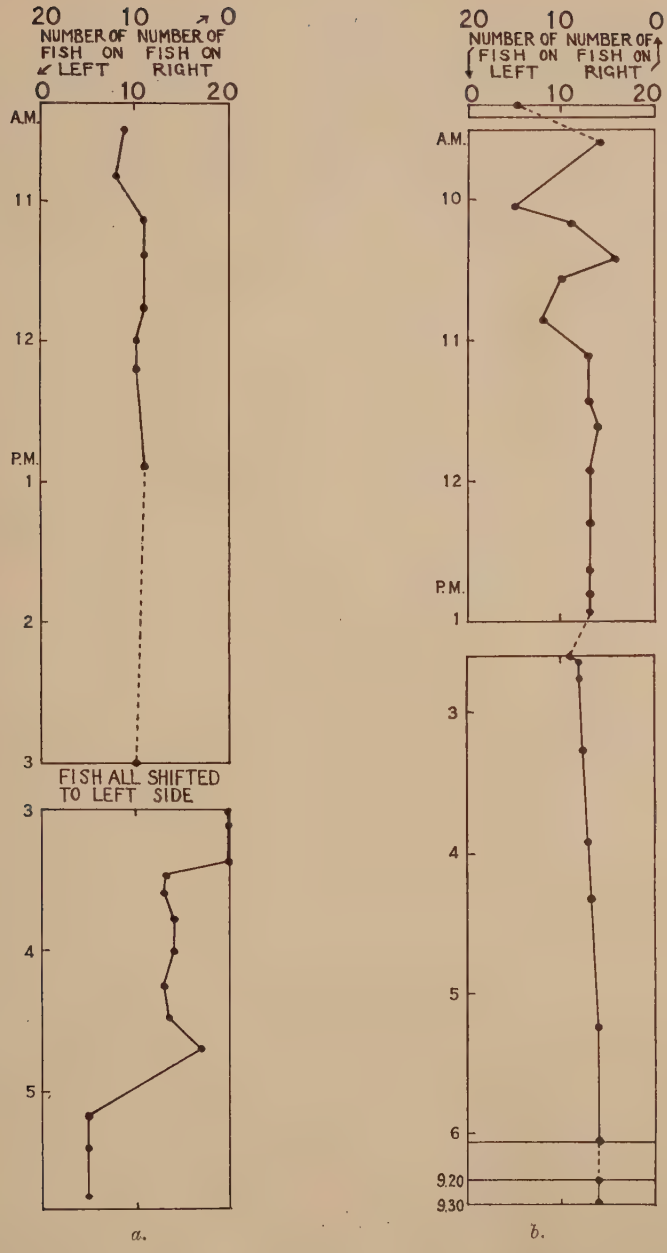

FIG. 11.—Graphs showing the distribution of a party of 20 or 21 fish in the two halves of a tank arranged as in Fig. 10. Two examples of the normal type of distribution.

The Main Attributes of That which induces a Schooling Response.

A bass is attracted towards another of its kind separated by a glass partition; it is attracted by its own reflection in a mirror. It may be asked, what are the essential characters in the object of attraction as

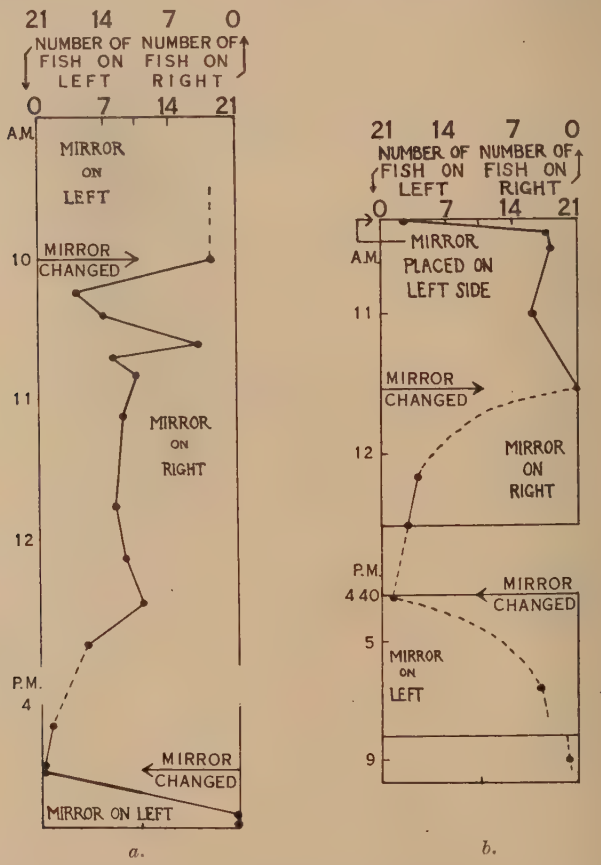

Fig. 12.—Graphs showing the distribution of a party of 20 or 21 fish in the two halves of a tank arranged as in Fig. 10. The effect of placing a mirror on one side and periodically changing it.

distinguished by the fish? Even granted that it is the "general appearance" as a whole which is significant, it may still be asked what are the main elements which contribute to the general resemblance—whether form, or movement, or pattern.

It was found that the fish will be attracted to, and lie alongside, a dead fish supported by glass in the position of a resting live fish.

The tank was fitted as shown in Figure 9. Two fish were in front of the glass. A dead fish supported in a glass jar was placed in the position ("MODEL") previously occupied by a mirror to which the fish had reacted well. (The tank was subjected to considerable disturbance while the arrangement was being fitted up.)

26.x.29	Mirror in position	Fish in region before mirror.
28.x.29 10 a.m.	Dead fish in place of mirror	Fish quiet in region of CD } ? Interval
11.45	" " " "	" " " " } while
12.20	Mirror in front of dead fish	} effects of
12.45 } 1.0 }	" " " "	Fish not in front of mirror } disturbance wore off.
2.10	Mirror removed, dead fish exposed to view	Both fish by mirror, but disturbed by the removal of it.
2.30	" " " "	Fish in region of CD, not by dead fish.
2.59	" " " "	Both fish lying up against dead fish, all facing in the same direction.
2.59 to 4.5 continuously }	" " " "	All this time the fish were lying up against dead fish, quiet. Sometimes orientated in some direction, sometimes in opposite, sometimes both differently.
4.15 to 4.32	" " " "	Fish away from dead fish in L. centre of the tank.
4.43	" " " "	At least one fish by dead fish.
5.20 } 5.23 }	" " " "	Both fish lying by dead fish.
29.x.30 9.5 } 10.0 }	" " " "	Through this period the fish were lying against dead fish, all orientated in the same direction.
10.10	Dead fish removed.	

On another occasion a similar experiment was repeated with similar results.

This shows clearly enough that the fish are attracted by the form of another fish, although it is completely devoid of movement. Hence movement of fins, for instance, is not an essential item.

Following on the experiment outlined above, several kinds of rough models were tried. One type bore the exact shape of the fish and was painted green, another of plasticene with a roughened surface, another a piece of bent tinfoil shaded dark in the upper half. The fish were attracted by none ; when they were too conspicuous the fish were definitely repelled. And it is scarcely surprising, for however much the models resembled fish in the air, under the water they stood out all too crudely. Little success was attained in getting a uniform lighting and still less in avoiding a harsh contrast with the background.

Still, it is at least an indication that the fish require an object that closely resembles a fish, the general shading and contrast between it and the background being important.

Reaction to Mirrors used as a Basis for further Experiments.

The fact that a fish will react, in a manner indicated above, towards other fish like itself might provide a starting-point for experiments to determine how far variation in the properties of the object to which reaction is made affects the results. For instance, what is the effect of cutting down the size of the space through which the object—whether another fish or mirror reflection—is viewed ? How much of the fish or image is necessary ?

Unfortunately the reactions of the fish to each other and to a mirror are not sufficiently cut-and-dried to provide a basis on which accurate comparisons can be drawn. It has been seen that the reaction of an individual fish to a plain mirror may vary considerably. For any given fish it is impossible to predict definitely how it will behave, but it is possible to say how it will most probably behave or the probable intensity of its reaction, especially if its condition is not abnormal. But it is not possible to measure this probability. Hence, in comparing the behaviour of an individual fish to a less efficient mirror or a less complete image, the comparison cannot be expressed at all accurately. All that can be said is that " the reaction on the whole appears less intense in this case than that " or " the probability for a certain reaction appears less in this case than that."

The nearest approach to a definite form of behaviour is seen in the case of a single fish in the presence of a mirror. It is strongly attracted to the mirror and spends much time in front of it. It is possible, therefore, to compare, as is attempted below, the behaviour of the fish towards various sizes of mirror exposure or various types of broken surface. But allowance must be made for greater variation in behaviour to less definite* situations

The method whereby the distribution of a number of fish gives statistical comparison (see pp. 441 and 442) does not lend itself for more refined application. It is adequate for showing the difference between " mirror " and " no mirror," but a glance at the figures is enough to show what little scope there is for expressing finer distinctions.

One attempt was made with the mirror covered over with black paper except for a slit $\frac{1}{2}'' \times 10''$. This had no effect on the grouping of the fish.

Consideration of such a statistical method shows that, even if the school is large enough (which it isn't) to eliminate individual variations, there is no reason why the mean value of the " state " of the fish should remain a normal constant. If the " state " of one fish changes, it is very likely that other fish for the same reasons will be changing in the same way. Hence the mean value, as it were, will shift. It must also be remembered that

* i.e. situations in which there is less contrast.

reaction to a mirror belongs to those set of reactions which keep the school together. Hence both reactions will interfere with each other ; particularly will the presence of other fish decrease intensity of reaction to the mirror.

Reaction of fish to small areas of mirror.

With a view to obtaining evidence on how complete an image is necessary to bring out the kind of behaviour described in earlier paragraphs, the fish were presented with exposures of mirror of various sizes. These were obtained by covering over a mirror with black paper out of which a piece of the required dimensions had been cut. The reflections of the fish, therefore, came to view as though through a gap in a black upright partition.

The results of observations are summarised as follows :—

	Whole Mirror.	Section $\frac{1}{2}$ in. × 10 in.	Whole Mirror.	Section $\frac{1}{4}$ in. × 10 in.
Small fish K, L, & O	Reacted well.	One fish reacts strongly, but is more attracted by the other fish.	Reacted well.	No reaction at all : apart from certain slight changes in their actions which might be the result of having detected movement.

	$\frac{1}{2}$ in. × 10 in.		1 in × 10 in.	1 in. × 2$\frac{1}{2}$ in.
	Various individuals have been observed to react to this slit, but has no statistical effect on a group of fish.			
Fish M 6$\frac{1}{4}$ in.	Small fish in tank as well. Reacted to reflection, but more attracted by young fish.	Alone. Reacted fairly well and lay up against it for some time.	Reacted as to full mirror.	Reacted very well. A good contrast in behaviour towards a similar exposure of plain glass.

	1 in. × 1 in. Exposure.
Fish N about 6 in.	Though watched over several days, never reacted at all.

As far as these tests go, it is clear that a complete image is not necessary, but it seems that it must be nearly complete. An eye alone or the head alone appears to exert no attraction. Furthermore, it must be remembered that the measure of the size of the exposure is no measure of the dimension of the image : when the eye is close to the aperture the field of view is extensive.

Reactions to a broken mirror surface.

One or two observations were made on the reactions of individual fish to a broken area of mirror surface. The mirror was covered with elastic bands, $\frac{1}{4}$ in. wide, spaced at definite intervals, running both across and along the surface. This gave a meshwork with small sections of mirror showing in between.

The following results were obtained from three small active fish (K, L, and O) which reacted well to a full mirror.

	Bands $\frac{3}{4}$ in. apart.	Bands $\frac{3}{8}$ in. apart.	Bands $\frac{1}{4}$ in. apart.
3 small fish, K, L, & O.	Definite reaction and fish spent some time lying against it.	Some sort of reaction but it was weak. Not attracted.	Scarcely reacted. Not attracted.

Another test showed that a number of fish will group in front of a mirror with a $\frac{3}{4}$-in. meshwork in the same way as in front of a partition of plain glass with similar meshwork through which other fish are visible beyond.

Experiments with banded mirrors were not continued because it became obvious that the results obtainable, unless possibly continued for an inordinately long time, were vague and of little value. When the bands were $\frac{1}{4}$-in. apart it could be seen that the fish on passing exhibited slight changes in their behaviour actions, and since bass are very sensitive to movement in the visual field it is not unreasonable to suppose that the movement of their reflection had effect on them. Movements of this kind are apt to attract the fish, no doubt because they are associated with "food situation." At what point is one entitled to distinguish between this type of attraction and that which produces a schooling response ? One must pay attention to those reactions which are different in the two situations, i.e. in one case there is the tendency to remain near or lie still against the source of attraction, in the other case there is none. When the whole reaction becomes so indefinite that these actions do not appear for certain, distinctions can no longer be drawn.

III. APPLICATION OF RESULTS TO THE SCHOOLING PROBLEM.

We have now to consider in what respects the data so far obtained bear on the problem of school formation for the particular case of the bass, and may therefore summarise them under the headings set down in the introduction (p. 423).

1 (*a*). With the bass, we have not been concerned with maintenance of school formation by a party of active fish moving from place to place. Within the confines of a laboratory tank schooling phenomena were limited to the grouping together of the few individuals under observation, particularly when in a resting or not very active state. The actions of individuals, however, which bring about the grouping in a tank, are at least connected, if not identical, with those which concern school formation under natural conditions.

The following are the kinds of actions which were observed. Movement towards other individuals (or one individual) up to within a certain short distance ; movement towards and stopping close besides resting

individuals ; the pursuit of another individual on the move ; orientation in the same direction as other individuals, either moving or resting ; change of direction of movement following change in the course of another fish ; movement instigated at the appearance of a moving fish ; speed of movement adjusted to that of other fish.

(*b*) The aspect of the environment concerned in these actions involves some kind of visual image in which undoubtedly shading, shape, and other spatial relations are distinguished. For evoking schooling behaviour movement is not essential, and a complete image is not essential.

(*c*) Results are sufficient to show that sight is the predominant, if not the only, sense involved ; but no experiments in sense physiology have been made to find what visual distinctions are most appreciated or what degree of refinement is exhibited.

2. It has been seen how bass in a tank are prone to group together, and, if separated, to move towards each other.

(*a*) They group more readily when disturbed or alarmed, and least when hunting for food. Small 3- and 4-in. fish will group with larger 6–7-in. fish, but not the larger with the smaller.

(*b*) The behaviour is variable, particularly in intensity, and cannot be profitably expressed on the basis of a simple tropism or system of tropisms. Since it clearly varies with the state of the fish, prediction of readiness to school cannot be made unless the state of the fish can be described.

(*c*) Two fish placed in a tank by themselves will keep fairly well together, but, if moving about actively, or engaged in feeding, or when signs of depression set in, their movements may be all but independent of each other. Inducing schooling experimentally goes no further than placing a fish which has a natural tendency to school under just those conditions which are normally required for schooling behaviour !

My best thanks are due to Dr. E. J. Allen particularly for suggesting this line of work and for the readiness with which he has given his valuable help and advice during the course of it.

SUMMARY.

1. Among schooling fish, the younger stages of the bass (*Morone labrax*) provide an instance in which the schooling habit, though strong, is not stereotyped into fixed forms of behaviour.

2. Observations were made on individual fish in connection with their attraction for one another, a special point being made of observing their behaviour in front of a mirror.

3. The data so obtained are summarised in the light of their bearing on the problems of school formation.

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The Validity of Single Vertical Hauls of the International Net in the Study of the Distribution of the Plankton.

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With 2 Figures in the Text.

Plankton studies, to be of direct application to fishery problems, should be of such a kind as to enable comparisons to be instituted between the fluctuations in a fishery and those in the plankton itself. But the collection of data on such a scale raises a whole host of difficulties, not the least of these being the degree to which a single haul of a vertical net—a method with much to recommend it—can be considered to yield a representative sample.

In 1922 the late Sir William Herdman (**1**), using a Nansen net, showed, for the waters off the Isle of Man, that " . . . conclusions drawn from any one haul might be anything up to 50 per cent wrong in either direction."

In 1925 Gardiner and Graham (**2**) described an experiment on the same lines, but using in this case a Petersen Young Fish Trawl, which net, they found, compared favourably with the so-called quantitative net employed by Herdman. But, in both cases, the fact emerged that the catches of successive hauls in the same limited area differed considerably from one another, the spread measured by the Standard Deviation being in the nature of $\pm 50\%$ of the mean. In order to see whether this does represent a fair figure, two series of consecutive hauls made during 1926 have been examined. Both the collection of the material, and the subsequent enumeration were the work of Mr. R. E. Savage, to whom I am greatly indebted for permission to make use of them. My thanks are also due to Mr. Michael Graham and others of my colleagues at Lowestoft, and particularly to Mr. Edser of the Ministry's Statistical Section, to whom I am indebted for many helpful suggestions. The net used was the International Net (**3**), of No. 3 silk (60 meshes to the inch), fished vertically by the Wollaston method (**4**).

In the first experiment 40 hauls were made during the course of the night May 31st–June 1st, 1926, whilst drifting in an area centred on a position about 15 miles E.$\frac{1}{2}$N. from the Tyne (Lat., 55° 5′ N.;

Long., 1° 0′ W.). The wind was westerly and light (Force 2 on the Beaufort Scale = approx. 5 m.p.h.), and the sea smooth. Soundings varied during the experiment, the nets being fished from 62–80 metres to the surface. The first haul was made at 1755, and the last at 0951, G.M.T. The duration of each haul, in seconds, was measured by means of a stop-watch.

In the forty hauls copepoda comprised from 70–95% of the total catch, the mean being 86%. The dominant species were *Pseudocalanus elongatus* and *Paracalanus parvus*, which for our present purpose have not been separated. The next two species in order of abundance were *Temora longicornis* and *Calanus finmarchicus*. Of non-copepoda, the only species that occurred regularly in fair quantities were Oikopleura spp., but the numbers were not large.

The second experiment comprised 38 hauls of the International Net made some three weeks later during the night of June 24th–25th, 1926, in an area centred on a position about 16 miles E.$\frac{1}{2}$N. from the Tyne (Lat., 55° 4′ N.; Long., 0° 57′ W.). The wind was northerly and stronger than before (Force 3 on the Beaufort Scale = approx. 10 m.p.h.). The sea was "slight to moderate" (3–4). Soundings remained fairly constant, and all hauls were made from a depth of 70 metres to the surface. The first haul was made at 2023, and the last at 0750, G.M.T.

Copepods were both absolutely and relatively more abundant, comprising about 94% of the plankton captured by the net. Oikopleura spp. were no longer common. Sagitta spp. were regularly present, but the numbers again were not large.

For the enumeration of the catch in the Laboratory, a Hensen Stempelpipette was used, and two, or more, separate samples were counted in the majority of cases, the factor being, usually, 50.

The numbers per metre depth of the selected organisms are set out in Tables I and II. The time at which each haul was made (in G.M.T.), the depth from which the net was fished, and the speed of hauling expressed as seconds per metre are included. For convenience in studying the tables, those observations lying close to one another in time are enclosed by brackets.

Before these data can be applied to the question before us, namely, to determine the reliability of a single haul of a vertical net, it is necessary to examine the conditions under which they were obtained. Both series of successive hauls were made from a drifting ship, and the time interval between the first and last haul was in one case about 16 hours, and in the other about 12 hours. There is no information as to the area of sea surface covered during the course of these experiments, but it must have been considerable. Further, owing to the wind, under the influence of which the vessel would tend to move independently of the main body of water, and to changes in level of the various species of the plankton, due

TABLE I.

First Experiment, May 31st/June 1st, 1926.

Times, Speeds, and Numbers per metre depth of Selected Organisms.

Haul No.	Time.	Depth.	Speed secs/metre.	Calanus.	Temora.	Pseudo-calanus + Para-calanus.	Total organisms.
1.	1755	80	1·3	6	21	12	50
2.	1805	80	1·1	14	37	22	80
3.	1820	80	2·0	12	29	27	80
4.	1825	80	1·9	2	9	12	46
5.	1940	80	1·8	18	26	32	93
6.		80	1·7	13	28	47	104
7.	1950	80	1·4	6	24	17	65
8.	2058	80	1·8	13	28	25	80
9.	2310	62	2·3	8	23	16	72
10.	0014	62	2·7	7	15	31	65
11.	0139	65	2·5	11	24	22	69
12.	0225	65	2·1	17	27	25	82
13.	0320	70	1·7	14	41	36	118
14.	0415	70	1·7	7	26	65	112
15.	0510	80	1·4	11	20	23	82
16.	0620	60	2·1	16	34	30	92
17.	0627	80	1·5	27	50	35	134
18.	0630	80	1·9	15	25	24	72
19.		80	1·8	20	45	29	110
20.	0647	80	2·2	32	62	61	180
21.	0650	75	2·0	38	44	42	142
22.	0655	75	1·5	5	32	27	85
23.	0700	80	1·7	28	27	51	120
24.		*80**	1·7	8	30	31	80
25.		*80*	1·7	35	38	57	168
26.		80	1·6	10	23	33	79
27.		80	1·7	7	21	16	51
28.		75	—	23	33	29	93
29.		80	1·7	13	25	24	70
30.		75	1·8	9	20	19	54
31.		*75*	—	7	29	23	65
32.		70	1·6	17	32	30	97
33.	0908	65	1·8	10	20	25	72
34.	0912	65	1·6	5	13	18	46
35.	0916	65	1·7	8	37	55	130
36.	0923	65	1·6	8	23	28	78
37.	0929	65	1·6	12	14	36	79
38.	0934	65	1·5	11	16	30	66
39.	0939	65	1·6	6	17	26	76
40.	0951	65	1·6	6	25	19	58

* Depths in italics are interpolated, the actual depths having been omitted from the records.

TABLE II.

Second Experiment, June 24th/25th, 1926.

Times, Speeds, and Numbers per metre depth of Selected Organisms.

Haul No.	Time.	Speed secs/metre.	Calanus.	Temora.	Pseudo-calanus + Para-calanus.	Total organisms.
1.	2023	1·9	56	22	47	146
2.	2200	1·6	69	13	61	174
3.	2205	1·8	46	15	55	198
4.	2220	1·8	37	8	84	141
5.	0125	2·1	36	19	47	112
6.	0130	1·9	25	15	66	122
7.	0137	1·9	98	44	159	355
8.	0145	1·9	64	38	51	219
9.	0155	2·0	58	20	112	232
10.	0245	2·0	92	15	111	261
11.	0254	2·1	139	26	132	332
12.	0305	1·9	139	28	142	359
13.	0315	2·2	95	31	193	380
14.	0323	2·0	155	50	134	379
15.	0350	2·0	95	36	169	352
16.	0355	2·0	159	61	139	444
17.	0402	2·3	156	43	206	492
18.	0408	2·3	150	49	208	496
19.	0415	2·2	166	57	237	544
20.	0420	2·1	91	46	229	406
21.	0426	2·3	116	39	—	446
22.	0433	2·3	101	43	161	367
23.	0500	2·1	89	46	73	246
24.	0507	2·0	111	59	77	275
25.	0515	2·3	72	61	102	279
26.	0520	2·1	90	30	89	265
27.	0525	2·1	108	45	154	356
28.	0532	2·3	119	47	—	308
29.	0535	2·3	151	53	132	384
30.	0645	2·3	104	44	192	394
31.	0700	2·3	101	71	154	352
32.	0708	2·4	146	84	231	539
33.	0712	2·4	113	74	199	458
34.	0720	2·4	115	62	137	365
35.	0730	2·4	111	58	234	461
36.	0736	2·4	143	44	169	404
37.	0745	2·2	114	50	151	381
38.	0750	2·4	102	47	164	391

to their own vertical migrations, it would be unwarrantable to assume that the same population has been sampled throughout the course of either experiment. Accordingly, to consider either series of experiments as a unit for subsequent statistical treatment would be to produce, in all probability, a false idea of the variation in numbers of individuals caught in successive hauls, and would tend unduly to lessen the degree upon which a single haul of a vertical net is to be relied. For this reason, then, although the full data for each experiment are given in Tables I and II, a certain proportion only has been utilised. The choice has been regulated as follows : No group of observations covers more than 1 hour ; no hourly group comprises less than 5 observations ; the observations within any group are not separated from one another by more than 12 minutes.

If Tables I and II are examined it will be seen that there are considerable variations in the catches of successive hauls, even where the time interval is short. The extent of this variation is most conveniently given by determining the mean number of individuals caught in groups of successive hauls, selected as above, and expressing the departure of each observation from this mean as a percentage deviation. These groups are shown in Tables I and II enclosed in brackets. The mean number of individuals captured in each group of observations has been called throughout the "group mean." In order readily to compare the extent of the variation within any one group with that in another, the Average Deviation within each group has been calculated.

The selected data treated in this way are set out in Tables III and IV.

It should be noted at this point that the whole of the variation between catches of successive hauls has apparently been assigned to the variability of the organisms themselves, and not to errors of the methodique. As will be seen later, this is in the main correct, the effect of such contributory errors as variations in the speed of hauling, the part played by light and darkness on the catching power of the net, and the magnitude of the enumeration error, being insufficient to account for the variation observed. The evidence in support of this is collected in an appendix, since by reason of its bulk it might otherwise appear to be the main burden of this paper.

By treating the data in such a way that the variations in the numbers of the more abundant species caught within periods of not more than one hour's duration are related to the mean of the period—the group mean—the observations are directly comparable one with another despite the fact that the density of population may be quite different. In this way, too, it may be possible to utilise observations from the two experiments as a unit for statistical treatment, if it can be shown that the degree of variation within the several groups is of about the same order of magnitude. Further, it may even be possible to consider all the species together.

TABLE III.

First Experiment. Percentage Deviations of Selected Observations from the Means for the Periods in which the Observations were made, and Average Deviation for the Period.

Haul No.	Times.	Percentage Deviations from Group Means of:— Calanus.	Temora.	Pseudo- + Paracalanus.
16		30	15	19
17		17	25	5
18		35	38	35
19	0620	13	13	22
20	to	39	55	65
21	0700	65	10	14
22		78	20	27
23		22	33	38
Group Mean		*23*	*40*	*37*
Average Deviation		37%	26%	28%
33		25	5	17
34		38	38	40
35		0	76	83
36	0908	0	10	7
37	to	50	33	20
38	0951	38	24	0
39		25	19	13
40		25	19	37
Group Mean		*8*	*21*	*30*
Average Deviation		25%	28%	27%

N.B. The Group Mean from which the percentage deviations have been calculated is shown in italics.

TABLE IV.

Second Experiment. Percentage Deviations of Selected Observations from the Means for the Periods in which the Observations were made, and Average Deviation for the Period.

Haul No.	Times.	Percentage Deviations from Group Means of:— Calanus.	Temora.	Pseudo- + Paracalanus.
5		36	30	46
6	0125	55	44	24
7	to	75	63	83
8	0155	14	41	41
9		4	26	29
Group Mean		*56*	*27*	*87*
Average Deviation		37%	41%	45%

Haul No.	Times.	Percentage Deviations from Group Means of:— Calanus.	Temora.	Pseudo-Paracalanus. ±
10		26	50	22
11	0245	12	13	7
12	to	12	7	0
13	0323	23	3	36
14		25	67	6
Group Mean		*124*	*30*	*142*
Average Deviation		20%	28%	14%
15		26	23	12
16		23	30	28
17		21	9	7
18	0350	16	4	8
19	to	29	21	23
20	0433	29	2	19
21		10	17	No observation
22		22	9	17
Group Mean		*129*	*47*	*193*
Average Deviation		22%	14%	16%
23		16	6	30
24		5	20	27
25	0500	32	24	3
26	to	15	39	15
27	0535	2	8	47
28		12	4	No observation
29		42	8	26
Group Mean		*106*	*49*	*105*
Average Deviation		18%	16%	25%
31		14	16	14
32		24	38	28
33		4	21	11
34	0700	3	2	24
35	to	6	5	30
36	0750	21	28	6
37		3	18	16
38		14	23	9
Group Mean		*118*	*61*	*180*
Average Deviation		11%	19%	17%

In order to answer these points, the averages of the deviation within each group have been collected together in Table V, and the values examined from the following points of view :

(*a*) Does any one species exhibit throughout a greater tendency to vary than any other ?

(*b*) Is the degree of variability greater during any one period ?

(*c*) Is the degree of variability more marked in either experiment ?

TABLE V.

COMPARISON OF SPREAD, EXPRESSED AS AVERAGE OF THE DEVIATIONS OF THE SELECTED OBSERVATIONS FROM THE GROUP MEANS.

Species.	Spread within each of the selected periods in the two experiments.							
Calanus	1st Experiment :	37%	20%	37%	22%	18%	25%	11%
	2nd Experiment :							
Temora	1st Experiment :	41%	28%	26%	14%	16%	28%	19%
	2nd Experiment :							
Pseudo-+ Paracalanus	1st Experiment :	45%	14%	28%	16%	25%	27%	17%
	2nd Experiment :							

It does not appear that one species exhibits greater variability than another.

During the first period of the Second Experiment, however, the Average Deviations are all bigger than obtained during the course of the remainder. They only differ, however, to a very small extent from those obtaining during the course of the First Experiment. On this account, therefore, there would seem to be no grounds on which any group of observations can legitimately be excluded. Nor does it appear to be unreasonable to treat all the observations as a unit for statistical treatment.

The problem may now be considered as follows : We have 145 attempts to obtain a measure of populations whose true values are given to us by the mean number captured in from 5–8 consecutive hauls of a vertical net. That is to say, we have sampled the population below a small area of the sea surface a number of times by a uniform method, and have determined the amounts by which any single haul differs from the mean of 5–8 hauls. This mean is taken, *faute de mieux*, to represent the actual number of organisms present, and is, in all probability, a close approximation to it. The same result would have been obtained had a special cruise been made, where, instead of the usual single haul at each station, a number of hauls at close intervals of time were taken, and the mean number of individuals used to give the density of population at each station. In view of the variability which has been shown to exist between successive hauls it would probably be desirable always to make up to 5 hauls at each station visited, but to do so would be to reduce the number of stations that could be visited in the course of a routine cruise.

If, however, the chance of a single haul giving a value within so much of the mean can be determined by some such method as that described above, we are enabled to state, with some degree of certainty, whether the resulting picture of the distribution of the plankton is more likely than not to be an accurate one.

In the two experiments under discussion we have been enabled, by the method adopted, to determine what proportion of our 145 observations lie within any desired amount of the mean. The point that arises now is to decide what latitude shall be allowed. To place the standard too low, and to say, for instance, that only those hauls lying within $\pm 10\%$ of the mean will be considered true values, is probably to aim at a degree of accuracy which, it is felt, is neither possible nor necessary. On the other hand, a standard placed at, say, $\pm 50\%$ allows, perhaps, too much latitude, and accordingly it has been placed at 33%. That is to say, any values lying within plus or minus 33% of the mean (or true) value, will be considered as near the truth as is necessary, taking into consideration their mode of collection, enumeration, and the use to which they are put.

The number of observations lying within 10%, 20%, 33% of the mean and the number above this amount are set out in Table VI.

TABLE VI.

FREQUENCY DISTRIBUTION OF PERCENTAGE DEVIATIONS (SIGNS IGNORED) OF ALL OBSERVATIONS FROM BOTH EXPERIMENTS.

Groups. %	Frequency.	Cumulative Frequency.	Percentage Cumulative Frequency.
0–9	33	33	23
10–19	33	66	46
20–33	46	112	77
34–39	13	125	86
40–49	7	132	91
50–59	4	136	94
60–69	4	140	97
70–79	3	143	99
80–89	2	145	100

It will be seen that of our 145 observations 112, that is 77 out of 100, lie within 33% plus or minus of the mean. Of the remainder, 13 out of 33 are within $\pm 40\%$. Thus, in the area where the two experiments were carried out, it is to be expected that, approximately, four out of every five hauls would give a value which for all practical purposes may be considered a true one. There is, however, the chance of getting values outside 33%, which may be termed extreme values, and, further, that

such extreme values as $\pm 90\%$ and even greater may be expected occasionally.

This result shows only limited agreement with that of Herdman (*loc. cit.*), and the question whether the degree of variability in the area where

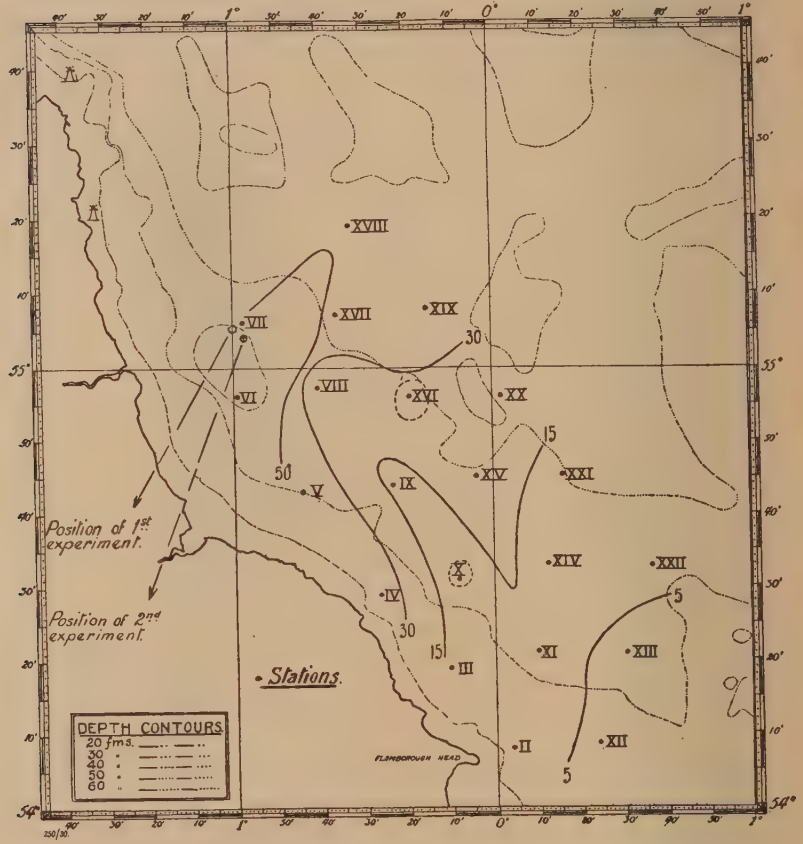

FIG. 1.—Position of Stations on Cruise 28 and Distribution of *Calanus finmarchicus*.

Roman numerals are station numbers. The distribution of Calanus is shown by contours according to the numbers per metre in a vertical haul. The observations of Stations X and XVI have been disregarded (see text, p. 461).

the experiments were performed *is peculiar to the area* must now be examined. In order to do so, the results obtained from a routine plankton cruise have been examined. The cruise selected (Number 28) was made immediately on the completion of our second series of experiments, and extended from June 26th–29th, 1926. At each of the twenty-one stations

a haul was made with the International Net (No. 3 Silk), fished vertically; horizontal hauls at 10-metre intervals from the surface to the bottom, with the same net, were taken at the eleven even-numbered stations, II, IV, VI, etc., and hauls of the Petersen Young Fish Trawl, fished obliquely for 30 minutes from (near) the bottom to the surface, were taken at the ten odd-numbered stations, III, V, VII, etc.

The position of the stations worked is shown in Figure 1. It will be seen that they lie on four lines roughly parallel to the coast. The numbers of one species (*Calanus finmarchicus*) per metre depth caught in the 21 hauls of the vertical net are given in Table VII.

TABLE VII.

NUMBERS PER METRE DEPTH OF CALANUS TAKEN IN VERTICAL NETS ON THE ROUTINE CRUISE OF JUNE 26TH–29TH, 1926, AND MEAN NUMBERS AT EACH STATION OF HORIZONTAL HAULS AT 10-METRE INTERVALS.

	CALANUS.	
Station No.	Nos. per metre vertical.	No. per Haul horizontal.
II	8	715
III	13	
IV	39	7,560
V	33	
VI	72	42,484
VII	46	
VIII	24	3,167
IX	12	
X	3	4,791
XI	10	
XII	3	820
XIII	1	
XIV	8	1,100
XV	27	
XVI	2	4,675
XVII	39	
XVIII	40	9,788
XIX	38	
XX	19	3,306
XXI	8	
XXII	8	750

If this Table is studied in connection with Figure 1, it will be seen that the numbers of Calanus increase as we go from south to north, reaching a maximum at Station VI. The species remains fairly abundant at Stations VII and VIII, but declines thence as we go again to the southward. Turning now at Station XIII, and running northwards further offshore, the numbers rise with one exception till Station XVIII is reached. Going south again from here, Station XIX, the second on our last line, shows large numbers of Calanus, but further south the numbers decrease steadily. An attempt has been made to "contour" the distribution of the species (see Fig. 1), and it is obvious that Calanus is *apparently* more abundant both near the coast and in the northernmost water of the area.

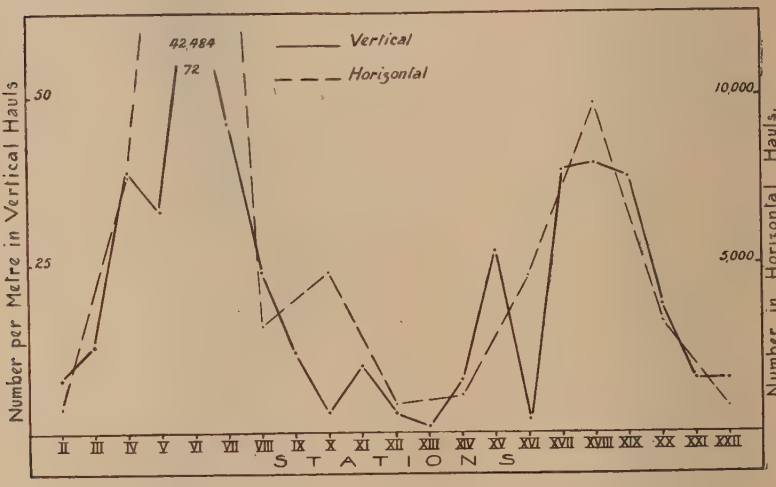

Fig. 2.—Numbers of Calanus taken in Vertical and Horizontal Hauls, Cruise 28.

sampled. This is shown equally clearly by Figure 2, which represents the numbers per metre of Calanus at each station, the stations being arranged in numerical order.

The unbroken line represents the numbers of Calanus captured in 21 vertical hauls. This gives, of course, the same apparent distribution as our contour. The chance that this distribution is a true picture is considerably enhanced by the continuity, and it can scarcely be the result of sampling an approximately uniform population by an unreliable method, or due to the fortuitous operation of chance in a population with no real geographical distribution.

The dotted line in Figure 2 represents the catches of eleven series of horizontal hauls with the same net at the even-numbered stations. This curve follows that of the vertical hauls so closely as to leave very little

room for doubt that the distribution is no more to be considered "apparent," but is a true picture.

Further, very slight confirmation of this is given by the 10 hauls of the Petersen Young Fish Trawl at the odd-numbered stations. The mean number of Calanus taken in the 30-minute oblique haul was only 385, whereas the mean for the horizontal hauls with a net of mouth area considerably smaller was 7196, showing that by far the greater proportion of Calanus must pass through the meshes of the fish trawl. In general, the catches of Calanus with this net are so small as to be of little value, but it is interesting to record that the biggest catch (2160) occurred at Station VII, which is in our area of greatest concentration.

It may be claimed, then, for this one cruise, that the distribution of Calanus, as given to us by single hauls of a vertical net at each station, lends support to a belief that the degree of variability in the area sampled is about the same as that in the smaller area where the two experiments were made.

The result of these two experiments, it will be recalled, was to show that only about 1/5th of the observations would give extreme values. Actually, on the cruise of 21 stations it is to be expected that 16 of the observed values would be within 33% of the truth, and that of the remaining 5 some would give values so far removed from it as completely to upset any attempt at contouring the observations. There are two such values at Stations X and XVI respectively. For both of these there is evidence from the horizontal hauls that the value is probably much too low (see Table VII), and there is, accordingly, good reason to believe that the contour should stand, approximately as drawn, the two discordantly low values being omitted. It is interesting to record that the contours were drawn in the first instance without the information which was derived later from the horizontal hauls, the two discordant values being omitted in view of the positions at which they occurred and the good continuity shown by the remainder, and because of the knowledge that such very discordant values were to be expected in, probably, not more than 2–3 cases. The evidence provided later by the horizontal hauls confirmed the omission of these two observations and tended materially to strengthen the belief in the criteria adopted.

In the course of examining the available information for this paper, the geographical distribution of Temora on this same routine cruise, and of Calanus and Temora on a preceding cruise was determined. (These data will be available shortly in a paper by Savage (**5**), and to save repetition are not reproduced here.) From a study of the contours produced, it would appear that the chance of approximately 4/5ths of the observations lying within 33% of the mean, and so providing values on which contours can be based, may be taken as a fairly safe guide.

We are now in a position to sum up both the results which have been obtained and their application to the question of the degree to which single hauls of a vertical net are reliable.

I. The distribution of the copepods Calanus, Temora, Pseudocalanus, and Paracalanus is such that consecutive vertical hauls of the International Net will not catch each time the same number of individuals. The greater proportion of any such hauls, however, will give values within $\pm 33\%$ of the mean (or true) value.

The actual proportion determined was 77 per 100, or approximately 4/5ths. Of the remaining 1/5th, nearly one-half will lie within $\pm 40\%$ of the mean, but extreme values up to $\pm 90\%$ and higher are to be expected.

II. The results of a routine plankton cruise during which 21 stations were worked in that part of the North Sea between 54° N. and 55° 20′ N., from near the coast to a line approximately 40 miles from it, suggest that probably no larger a proportion of extreme values obtains in this area, since the results can be contoured with the exception of 2 discordant values only.

III. The criteria which it is suggested should be adopted to enable the probability of accuracy of any distribution picture derived from the use of single hauls of a vertical net to be gauged are (*a*) the continuity of the observations themselves, by which is meant the mutual support lent to each other by the observations at adjacent stations, and (*b*) the knowledge that, probably, no less than 4/5ths of the observations will lie within $\pm 33\%$ of the mean.

IV. It appears that extreme values of $\pm 50\%$ and upwards of the mean are to be expected occasionally, but probably not more often than once in ten, and on this account a single high or low value unsupported by similar values at adjacent stations is meaningless.

V. Where there is no continuity (see III (*a*) above) it must be taken to mean that the species itself has exhibited no clearly defined area-preference.

VI. Subject to the limitations of III and IV (above), the use of single vertical hauls to study the distribution of the plankton in both space and time may be expected to give reliable results.

APPENDIX A.

SOURCES OF ERROR WHICH MAY ACCOUNT ENTIRELY, OR IN PART, FOR THE VARIATION IN THE SIZE OF CATCH OF THE SUCCESSIVE HAULS CONSIDERED IN THE FOREGOING SECTION.

In this appendix it is proposed to examine those sources of error of the methodique to which the observed variations may be due. The main sources of error will be :—

1. Variations in the speed at which the net was hauled through the water.
2. The effect of light and darkness on the catch of the net.
3. The enumeration error.

Other, minor, sources of error are :—

4. Losses due to insufficient washing of the net, accidents in bottling the sample, etc.
5. Mistakes in identification during enumeration.

The two last-named may safely be considered unimportant, but the effects of the first three may have been considerable, and it is with these that this section will deal.

1. *Speed of Hauling.*

Robert (**6**) working in fresh water with a Fuhrmann Net, No. 20 silk, showed that the numbers of certain zoo- and phyto-planktons captured were directly proportional to the speed at which the net was fished. In both our experiments the speed varied between successive hauls, and it is desirable to see the effect of this, if any, on the catches. In the second series, owing presumably to the stronger wind resulting in the vessel drifting more during the haul, the mean time the net took to pass through 1 metre of water was slightly longer than in the first (2·1 seconds per metre, and 1·8 seconds per metre respectively). This, coupled with the fact that the population had increased, does not allow us to treat the two experiments together, which would be desirable in view of the relatively small number of observations.

In the first series the time of hauling per metre varied from 1·1 to 2·7 seconds, with a Standard Deviation of $\pm 0{\cdot}306$. In the second case, the speed was more uniform, the range being from 1·6 to 2·4 seconds per metre with a sigma of $\pm 0{\cdot}187$. If a correlation exists between variations in the speed of hauling and in the numbers of individuals captured, it should appear in the first experiment, and it is this series that has been examined. The data selected for this purpose were the totals of the four dominant copepods, whose abundance in each of the 40 hauls of this series has been related to the speed (metres per second) at which each haul was made, and the coefficient of correlation (r) determined. The value of r from these data was found to be inconsiderable, namely, $-0{\cdot}13$. It is, accordingly, a legitimate conclusion that the variations in the numbers of organisms captured are due to some other cause than changes in the speed of hauling.

2. *The Effect of Light and Darkness on the Catch of the Net.*

This question arises in view of the fact that there is some evidence that certain species are taken, even in vertical hauls, in greater numbers

by night than by day. Whether this is due to the animals rising from those lowest layers which the vertical net does not sample, or whether, as suggested by Southern and Gardiner (**7**), certain forms can see and avoid a net by day, is outside the scope of this paper. But there is a possibility that the night catches may have differed in amount from those by day. Had it not been necessary, for the reasons given on page 453, to consider only those differences in the size of catch of hauls closely related to one another in time, the effect of light and darkness might have been considerable. As it is, it is unlikely that great changes in level are to be expected in the course of the short intervals of time separating the hauls within each group, and for the same reason it is not to be expected that changes in light intensity would be sufficiently great to influence the reaction of the organism towards the net.

In the first experiment, the selected catches were all made in daylight (see Table I). In the second experiment (Table II) the hauls of the first three groups were made at night, and those of the remaining two by day, the mean number of the selected copepods being as follows :—

Species.	Day.		Night.	
	No. of Hauls made.	Mean No. Captured.	No. of Hauls made.	Mean No. Captured.
Calanus	15	112	18	107
Temora	15	55	18	37
Pseudo- + Paracalanus	14	148	17	147

Apart from Temora, which is nearly 50% more abundant by day, the day and night means are to all purposes the same, and it is probably safe to assume that those species are not more abundant in hauls made at night, and that the possibility of this phenomenon vitiating our conclusions may thus be ignored.

The Enumeration Error.

It has long been recognised that a single sample withdrawn by means of a Hensen Stempel-pipette, and raised by the dilution factor, will not give the actual number of organisms present in the whole catch, and Hensen adopted a method of averaging to minimise the discrepancy, *vide* Jenkins (**8**). A large enumeration error might, of course, account for a great part of the differences that have been observed in the size of catches of successive nets, and, in order to gain an idea of the magnitude of the error when a single sample only is withdrawn for enumeration a series of test " counts " was made. One cubic centimetre was withdrawn, by means of a Hensen Stempel-pipette, 25 times from a stock sample of North Sea plankton, diluted to 200 c.c. After each " count " the organisms were

drained, replaced in the flask, and 1·0 c.c. of water run in from a burette. In this way the " population " was kept constant.

The results of these 25 " counts " are set out in Table VIII. To get the total number of each species actually present in the sample the counts should be multiplied by the factor 200.

TABLE VIII.

Results of 25 Test Counts.

Sample.	Calanus.	Pseudo- + Paracalanus.	Temora.	Total Organisms.
1.	39	51	11	115
2.	28	59	12	123
3.	38	47	16	131
4.	49	59	8	133
5.	43	60	17	134
6.	42	49	15	122
7.	51	57	12	138
8.	39	54	10	118
9.	41	44	13	115
10.	40	37	16	108
11.	36	59	8	118
12.	49	63	9	138
13.	38	42	7	103
14.	44	52	17	127
15.	36	50	16	113
16.	38	59	16	125
17.	39	60	13	127
18.	46	58	20	146
19.	43	65	11	135
20.	43	49	9	119
21.	40	61	9	122
22.	43	56	14	129
23.	38	60	13	125
24.	53	69	9	149
25.	34	57	9	119
Total	1,030	1,377	310	3,132
Mean	41·2	55·1	12·4	125·3
Mean × 200	8,240	11,020	2,480	25,060

To get a measure of the spread of this series of counts the Standard Deviations (σ) have been calculated, after raising each count by the dilution factor of 200, for the three copepod species and for all the organisms present. These are set out in Table IX.

TABLE IX.

Standard Deviation of 25 Test Counts.

(Given in Table VIII.)

Calanus.	Temora.	Pseudo- + Paracalanus.	Total Organisms.
1,088	1,472	690	2,179

TABLE X.

Comparison of 25 Hauls from the Second Experiment, Selected by Chance, with the 25 Test Counts.

Actual Hauls.			Test Counts.		
Haul No.	Total Calanus.	Percentage Deviation from Mean.	Count No.	Total Calanus.	Percentage Deviation from Mean.
29	10,575	+ 40·5	1	7,800	— 5·3
13	6,675	— 11·3	2	5,600	— 32·0
36	10,025	+ 33·2	3	7,600	— 7·8
20	6,360	— 15·5	4	9,800	+ 18·9
4	2,600	— 65·5	5	8,600	+ 4·4
23	6,225	— 17·3	6	8,400	+ 1·9
7	6,900	— 8·3	7	10,200	+ 23·8
34	8,160	+ 8·4	8	7,800	— 5·3
18	10,500	+ 39·5	9	8,200	— 0·5
2	4,845	— 35·6	10	8,000	— 2·9
25	5,400	— 28·3	11	7,200	— 12·6
9	4,050	— 46·2	12	9,800	+ 18·9
32	10,200	+ 35·5	13	7,600	— 7·8
16	11,100	+ 47·5	14	8,800	+ 6·8
35	7,800	+ 3·6	15	7,200	— 12·6
19	11,640	+ 54·6	16	7,600	— 7·8
3	3,240	— 57·0	17	7,800	— 5·3
30	7,275	— 3·3	18	9,200	+ 11·7
14	10,875	+ 44·5	19	8,600	+ 4·4
33	7,920	+ 5·2	20	8,600	+ 4·4
21	8,100	+ 7·6	21	8,000	— 2·9
5	2,520	— 66·5	22	8,600	+ 4·4
28	8,325	+ 10·6	23	7,600	— 7·8
12	9,775	+ 29·9	24	10,600	+ 28·6
31	7,080	— 5·9	25	6,800	— 17·5
Mean	7,527	28·85	Mean	8,240	10·25

The results cannot be considered altogether satisfactory, and the possibility that part, at least, of the variation in the size of catch may be due to the enumeration error cannot be ruled out. That the variation found is not due solely to this cause is demonstrated by the following table, where the variation in the numbers of Calanus captured in 25 hauls of the 38 consecutive hauls of the Second Experiment is compared with that of this series of 25 test counts. The 25 hauls in Table X, opposite, were selected from the total of 38 hauls of Table II, by dealing out the first 25 cards from a pack of 38 numbered 1–38, the choice being in this way unbiased.

It will be seen at once that the degree of variation in the numbers of Calanus caught in the 25 hauls, selected from the series of consecutive hauls of the Second Experiment, is much greater than that of the test counts. Further, it must be remembered that column 5 shows the situation at its worst, for in both the actual experiments the factor, instead of 200, was usually 50, and the enumerated totals were based on an average of 2 (and sometimes more) separate counts. Turning again to Table IX, we see that sigma for Calanus was ± 1088, while $\frac{100 \cdot \sigma}{m}$ is $\pm 13{\cdot}2\%$. If now we compute sigma for the twenty-five counts taken two at a time, sigma becomes ± 715, $\frac{100 \cdot \sigma}{m}$ becoming $\pm 8{\cdot}7\%$. It may fairly be claimed, therefore, that although the enumeration error may be considerable, it is not sufficient to account for the differences observed in our two series of successive hauls.

SUMMARY.

In both the series of hauls made for the purpose of estimating the reliability of a single haul of the International Vertical Net, the possibility that the variation in the size of catch of successive hauls is due to errors of the methodique has been examined.

Errors due to variations in the speed of hauling, and to the effect of light and darkness on the catch of the net can be ruled out.

The enumeration error, even where the enumerated total rests on a single count, is insufficient to account for the major part of the variations found, and, having regard to the fact that such totals were derived from two, and sometimes more, separate " counts " from each sample, there is good reason to believe that the main cause of variation in the size of catch is to be sought elsewhere, the most likely being the lack of uniformity in the distribution of the organisms themselves.

APPENDIX B.

VARIATIONS IN THE COMPOSITION OF THE PLANKTON DURING THE COURSE OF THE TWO SERIES OF CONSECUTIVE HAULS.

A STUDY of Tables I and II (pp. 451 to 452), which give the numbers per metre depth of the selected species in each haul, shows that there was considerable variation in the numbers of individuals caught, even where the time interval between the hauls was quite short. Whether this variation was ever sufficiently great to warrant the statement that the plankton was patchy, or that the individuals comprising it occurred in swarms, will depend upon the interpretation placed on these somewhat vague terms. Apstein (**9**), for instance, defines a swarm as a local assemblage of animals of one species in waters where, elsewhere, this species is absent or very sparsely distributed. It will be seen, however, that he regards a swarm as composed of one species. With this definition in mind, the data have been examined to see whether or not a population has been sampled that, whilst varying in abundance, maintained a fairly uniform specific composition. The method adopted has been to express the numbers of the dominant copepods as plus or minus percentage deviations from their own means, in which way it has then been possible to see whether the species tend to increase or decrease separately or together. The data treated in this way are set out in Tables XI and XII.

TABLE XI.

NUMBERS OF CALANUS, TEMORA AND OF PSEUDO- AND PARACALANUS PER METRE DEPTH IN 40 CONSECUTIVE HAULS FROM THE FIRST EXPERIMENT, EXPRESSED AS PERCENTAGE DEVIATIONS FROM THEIR OWN MEANS.

(Hauls in which the numbers of the three genera did *not* rise or fall together are in italics.)

Haul No.	Calanus.		Temora.		Pseudo- + Paracalanus.	
	+ %	− %	+ %	− %	+ %	− %
1.		57		25		61
2.	*0*	*0*	*32*			*29*
3.		*14*	*4*			*13*
4.		86		68		61
5.	*29*			*7*	*3*	
6.		*7*	*0*	*0*	*52*	
7.		57		14		45
8.		*7*	*0*	*0*		*19*
9.		43		18		48
10.		*50*		*46*	*0*	*0*

Haul No.	Calanus. + %	Calanus. −%	Temora. + %	Temora. −%	Pseudo- + Paracalanus. + %	Pseudo- + Paracalanus. −%
11.		21		14		29
12.	*21*			*4*		*19*
13.	*0*	*0*	*46*		*16*	
14.		*50*		*7*	*110*	
15.		21		29		26
16.	*14*		*21*			*3*
17.	93		79		13	
18.	*7*			*11*		*23*
19.	*43*		*61*			*6*
20.	129		121		97	
21.	171		57		35	
22.		*64*	*14*			*13*
23.	*100*			*4*	*65*	
24.		*43*	*7*		*0*	*0*
25.	150		36		84	
26.		*29*		*18*	*6*	
27.		50		25		48
28.	*64*		*18*			*6*
29.		7		11		23
30.		36		29		39
31.		*50*	*4*			*26*
32.	*21*		*14*			*3*
33.		29		29		19
34.		64		54		42
35.		*43*	*32*		*77*	
36.		43		18		10
37.		*14*		*50*	*16*	
38.		21		43		3
39.		57		39		16
40.		57		11		39

TABLE XII.

NUMBERS OF CALANUS, TEMORA AND OF PSEUDO- AND PARACALANUS PER METRE DEPTH, IN 38 CONSECUTIVE HAULS, FROM THE SECOND EXPERIMENT, EXPRESSED AS PERCENTAGE DEVIATIONS FROM THEIR OWN MEANS.

(Hauls in which the numbers of the three genera did *not* rise or fall together are in italics.)

Haul No.	Calanus.		Temora.		Pseudo- + Paracalanus.	
	+%	−%	+%	−%	+%	−%
1.		46		48		66
2.		34		69		56
3.		56		64		60
4.		64		81		40
5.		65		55		66
6.		76		64		53
7.		*6*	*5*		*14*	
8.		38		10		63
9.		44		52		19
10.		12		64		20
11.	*34*			*38*		*5*
12.	*34*			*33*	*2*	
13.		*9*		*26*	*39*	
14.	*49*		*19*			*4*
15.		*9*		*14*	*22*	
16.	*53*		*45*		*0*	*0*
17.	50		2		48	
18.	44		17		50	
19.	60		36		71	
20.		*12*	*10*		*65*	
21.	*12*			*7*	No observation.	
22.		*3*	*2*		*16*	
23.		*14*	*10*			*47*
24.	*7*		*40*			*45*
25.		*31*	*45*			*27*
26.		13		29		36
27.	4		7		11	
28.	*14*		*12*		No observation.	
29.	*45*		*26*			*5*
30.	*0*	*0*	*5*		*38*	

Haul No.	Calanus. + %	Calanus. − %	Temora. + %	Temora. − %	Pseudo- + Paracalanus. + %	Pseudo- + Paracalanus. − %
31.		*3*	*69*		*11*	
32.	40		100		66	
33.	9		76		43	
34.	*11*		*48*			*1*
35.	7		38		68	
36.	37		5		22	
37.	10		19		9	
38.		*2*	*12*		*18*	

In the First Experiment (Table XI) all three of the selected species rise or fall together in 19 out of 40 cases. These are shown in ordinary type. In 15 of these cases the amounts are below the respective means, and in 4 they are above. Taken by itself, this is not perhaps very impressive, but the probability that it does indicate a tendency for the three species to behave similarly is heightened by a study of the figures themselves, which in several cases are of the same order of magnitude.

In one case, however, haul 14 (Table XI), the copepods Pseudo- and Paracalanus occurred in considerable numbers, whilst the numbers of Calanus and Temora showed a decrease, suggesting that here a definite aggregation of the species must have been sampled.

In the Second Experiment (Table XII), the three copepods rise or fall together on 19 occasions out of 36. On 10 occasions they have all fallen and on 9 they have risen together. Here again, particularly in hauls 1–6, Table XII, where the three species are all relatively scarce, the probability that the species have behaved in the same way is heightened by the similarity of the deviations.

In this series there is no evidence of a mono-specific swarm.

In conclusion, it would probably be unwise to attach too great weight to these somewhat scanty data, but it would appear that mono-specific aggregations are only exceptionally encountered, and that the population sampled has maintained a relatively constant composition.

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The Deterioration of Fabrics Exposed on a Roof after Treatment with Fishing-net Preservatives.

By

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Work on fishing nets showed that the rapid deterioration which they undergo in water is due to bacterial action and may be greatly reduced by the use of appropriate preservatives which hinder bacterial growth. Nets are however exposed to sunlight and to diffuse light also, as well as to the action of rain and wind. It seemed desirable, therefore, to see whether the preservatives found to be of use against bacterial action were also useful in affording protection against the weather and such a degree of moisture as normally occurs in fabrics thus exposed. Instead however of using nets, fabrics were used, as they give rather more uniform results than do nets, each strip tested being made up of many strands. Furthermore, previous experience with fabrics had shown how great their rate of deterioration might be in Egypt, where there is intense sunlight. For example, the linen used on aeroplane wings, when exposed untreated, is reduced to about half its initial tensile strength in fifty days during summer, and the stout fabric of which hangars are made may be reduced to between one-fifth and one-tenth of its tensile strength in ten months—this refers to the side facing south, the north side being far less acted on.

It was found that cotton resisted weathering better than linen. Neither cuprammonium treatment (Willesden canvas), treatment with bitumen, rubber proofing nor dyes availed to lessen the rate of deterioration. Proofing with basic aluminium acetate was however definitely better than no treatment.

Conditions in Plymouth are however rather different, the sunlight is less intense, and the fabrics remain wet for longer periods, so that one might expect the deterioration due to bacteria and moulds to be relatively greater than in Egypt, in spite of the lower temperature.

There was another object in view in undertaking these tests, namely, to ascertain whether treatment with the preservatives recommended for use on nets had any injurious action on their tensile strength when exposed to sunlight, either from the properties of the preservative or of such decomposition products as might arise in the course of weathering.

Exposure Tests.

The fabrics were exposed on the flat roof of the Laboratory. This faces the sea and from it come the prevailing winds. Even with other winds the air is not polluted nearly as heavily as in industrial towns; accordingly the effect of acid fumes on the fibres may be considered as of little importance, especially as there is enough driven spray from the sea during storms to neutralize traces of acid.

The fabrics were exposed after being stretched by hand on wooden frames of the dimensions used for such tests at the National Physical Laboratory; these permit of five two-inch strips, eight inches long, being cut both to test warp and weft, leaving a good margin from the edge so as to avoid any possible effects due to the wood. The frames were exposed horizontally.

The materials used were a thick cotton, sold in yard widths at almost three shillings a yard; a lighter cotton, same width, sold at twenty-two pence per yard, and a stout linen, same width, sold at twenty-eight pence per yard. The preservatives and their method of application are as previously given in the work on net preservation (1928). The results of the exposures are shown in the three tables. The tensile tests were carried out at the Royal William Yard, Plymouth, through the courtesy of the Superintendent and Mr. R. M. Filmer, F.I.C., Chief Chemist.

The tests were carried out upon strips 2 inches wide and $6\frac{5}{8}$ inches between the grips of the Goodbrand Machine under the standard conditions for such tests. The columns show the percentage strength of the strips at breaking, the breaks being clean. The figures are the mean of the five determinations in each case and are calculated on the values for the untreated, unexposed, controls.

TABLE I.

To show deterioration in a heavy weight cotton fabric, initial tensile strength No. 1, warp 166, weft 276 lbs. per inch, No. 25, warp 165, weft 150 lbs. per inch, thickness approximately 0·8 mm., exposed horizontally on the roof, Nos. 2–4, from 10/1/'25–10/1/'27, two years, Nos. 15–19, from 12/9/'25–18/11/'26, fourteen months, and Nos. 27–31, from 25/11/'26–25/3/'29, two years and four months. No. 25 is control for above from No. 15 onwards.

No.	Treatment.	Warp.	Weft.
1	None, unexposed	100	100
2	None, exposed	66	70
3	Dried and dipped in 10% copper oleate	62	62
4	Dried and dipped twice in 5% mixed copper soaps	62	63

No.	Treatment.	Warp.	Weft.
15	None, exposed	77	81
16	Dipped in 10% mixed copper soaps	70	79
17	Do.+$\frac{1}{4}$ lb. resin per gallon	73	82
18	Do.+$\frac{1}{2}$ lb. ,, ,,	68	77
19	Do.+1 lb. ,, ,,	71	85
25	None, unexposed	100	100
27	Dipped in Cuprinol	49	59
29	Dipped in 10% mixed copper soaps	62	75
30	Do.+2 oz. coal tar per gallon	65	77
31	Do.+4 oz. ,, ,, ,,	64	70

TABLE II.

To show deterioration in a light weight cotton fabric, initial strength, warp 124, weft 78 lbs. per inch, approximately 0·5 mm. thick, exposed horizontally on the roof from 10/1/'25 to 10/1/'27.

No.	Treatment.	Warp.	Weft.
9	Untreated, unexposed	100	100
10	Untreated	31	31
11	Dipped in 10% copper oleate	28	30
12	Do., but dried before dip	27	28
13	Dipped in 5% mixed copper soaps, dried previously	31	30
14	Dipped twice ,, ,, ,, ,,	28	31

TABLE III.

To show deterioration in a heavy linen fabric, approximately 0·7 mm. thick, exposed horizontally on the roof, Nos. 6–8 for two years, 10/1/'25–10/1/'27, Nos. 20–24 from 12/9/'25–18/11/'26, viz. 14 months, Nos. 32–36, exposed 25/11/'26–25/3/'29, two years and four months. No. 5 is control for Nos. 6–8 and had a tensile strength of 200 lbs. per inch in the warp and 178 lbs. in the weft. No. 26 is control for Nos. 20–24 and 32–36; it had a tensile strength of 170 lbs. per inch in the warp and 206 lbs in the weft.

No.	Treatment.	Warp.	Weft.
5	Untreated, unexposed	100	100
6	Untreated	40	54
7	Dipped in 10% copper oleate; previously dried	39	57
8	Dipped in 5% mixed copper soaps; previously dried	39	67

No.	Treatment.	Warp.	Weft.
20	Untreated, exposed 12/9/'25	55	62
21	Dipped in 10% mixed copper soaps	75	72
22	Do.+$\frac{1}{4}$ lb. resin per gallon	70	85
23	Do.+$\frac{1}{2}$ lb. ,, ,,	69	83
24	Do.+1 lb. ,, ,,	76	79
26	Untreated, unexposed	100	100
32	Cuprinol, one dip, exposed 25/11/'26	42	65
35	Mixed copper soaps, one dip	60	68
36	Do.+tar 4 oz. per gallon, one dip	54	66

DISCUSSION OF RESULTS.

In the first series of exposures, Nos. 1–14, it may be seen that the cotton (Table I) lost about one-third of its strength in the two years, the thinner cotton (Table II) over two-thirds, and the linen (Table III) 60 per cent in warp and 46 per cent in weft. In all three sets the copper oleate or mixed copper soap (oleate, stearate, palmitate) treatment gave final strengths very close to that of the untreated fabrics. It is therefore obvious that the deterioration was due to the weathering, especially the action of light, since the copper soaps protect against bacteria and moulds. Under such conditions the effect of the latter appears to be negligible. The soaps, however, have not hastened the weathering action.

In the second series, Nos. 15–26, the treated cottons (Table I) are slightly weaker in the weft, but the same as or stronger than the control in the warp. The linen (Table III) is better than the control in both warp and weft, though the difference is not large. The resin copper soap mixtures adhere better and so resist the leaching action of rain, though after 14 months the upper surfaces showed no visible effects of the treatment, though the lower surfaces did.

The third series was again a two years' exposure, like the first. The copper soap treated cotton, No. 29, was very similar in its behaviour to that in the first set, No. 4, whereas the linen behaved rather better in the third series, cp. Nos. 35 and 7. The addition of tar to the soaps was intended to improve their adherence, which it does, and to cut off the light, which it does not appear to do, as far as the surface is concerned, for any length of time, for Nos. 30 and 31 are only slightly better than Nos. 3 and 4, these being cotton. With the linen, Nos. 35 and 36, the preservation is rather better than in Nos. 7 and 8. Very strangely the Cuprinol-treated fabrics, both linen and cotton, are appreciably weaker than the fatty soap and tar mixtures. Cuprinol, which is a copper soap of a

petroleum acid, gives excellent protection against bacterial action and remains on the exposed fabric well, better than the fatty soaps alone.

None of the treatments tried, therefore, are really effective in lessening the destructive weathering action. Dr. R. H. Pickard, Director of the British Cotton Industry Research Association, kindly drew the author's attention to a method of preserving fabrics used at Woolwich, by means of precipitated chromium or chromium and iron hydroxides. The value of this treatment was made clear by work of Cunliffe and Farrow (1928), though they refer to Mr. J. R. Hannay for pointing out its value to them. It will be of interest to see whether it is not possible to combine this with the copper soap treatment. Possibly the chrome-green process alone may have an appreciable antiseptic action. In any case, it ought to be of value for sails and other fabrics exposed on a sea-going craft.

SUMMARY.

(1) Cotton and linen fabrics were exposed horizontally on the Laboratory roof at Plymouth for two years. During this time the fabrics lost from one to two-thirds of their initial tensile strengths in both warp and weft.

(2) The application of copper oleate, mixed copper soaps, also of the latter with resin or tar as binding agents, had no effect or a slight effect only in lessening deterioration: such weakening must therefore be due to weathering, chiefly the action of light, since these substances have been shown to be useful antiseptics on nets immersed in salt or fresh water.

(3) No deleterious action may be expected when nets treated with these substances are exposed to light, since the treated fabrics were no weaker than the untreated: but where Cuprinol had been used, there appears to have been some weakening, though the antiseptic properties of Cuprinol are excellent.

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Note on the Condition of the Water in a Marine Aquarium.

By

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Somewhere about 1914 D. J. Matthews maintained the sea-water in the Plymouth aquarium tanks at a pH value close to that of the outside sea-water by the addition of suitable quantities of sodium carbonate.

In 1922 the writer found that the aquarium tanks were usually at pH 7·6, whereas the water of the Sound was at pH 8·1 on an average and the open sea between pH 8·14 and 8·28. Any lowering of the value for the tanks denotes an abnormal excess of carbon dioxide, and when pH 7·3 is reached symptoms of distress may appear among the fishes ; water at pH 7·1 was found to be definitely foul and evil-smelling.

It was suggested that lime should be added to the tank water to remove the excess of carbon dioxide. This also serves to neutralise acid substances excreted by the animals. It was found that the addition of about 1·5 kg. of quicklime, mixed in a bucket of water, to the resting reservoir each week sufficed to maintain the reaction slightly over pH 8. There are two reservoirs, which are used in the circulation and allowed to sediment during alternate weeks. Though the fishes and many invertebrates throve in water at pH 7·6 the more delicate organisms did better when it was maintained at pH 8. With no addition of lime the water is oxygenated by splashing and by compressed air, and at the same time much carbon dioxide is removed. Additions of sea-water are made at infrequent irregular intervals when the Sound water is specially clear and clean. The addition of lime has now gone on since about 1923 with satisfactory results.

The method has however been criticised unfavourably by Breder and Howley (1930), who have also made statements concerning aquarium water which call for comment.

The fishes are said to produce carbon dioxide which " by hydrolysis forms carbonic acid (H_2CO_3) which tends to make the water more acid." The process is not hydrolysis nor does the water ever become acid, though its alkalinity is of course reduced somewhat.

It is further stated that " the continued excretion of acid metabolites

too removes carbon dioxide, though not quite as efficiently. weight, when in the form of crystals.

London Zoological Society's Aquarium, where filtration and the tank are used for purification, the buffering of the water somewhat reduced (Stowell, 1925, 1926; Brown, 1929).

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On the Colorimetric Determination of pH in Sea-Water.

By

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With 2 Figures in the Text.

It is not always possible accurately to carry out pH determinations on board ship, owing to bad light or other conditions, although it may be possible to collect water samples. Hitherto it has not been considered possible to determine pH more than a few hours after collection, owing to the action of bacteria and phytoplankton and the exchange of carbon dioxide with the atmosphere.

A method has now been found whereby the water may be kept in its original condition for some considerable time after collection, enabling the actual determination to be carried out under more favourable conditions in a shore laboratory.

Colorimetric estimation is made by matching the samples under examination with buffer solutions corresponding to known pH. Accurate comparison is dependent upon the quality of the light used, since the colour exhibited by an indicator is due to the selective absorption of certain frequencies of the incident light. An estimation made with sunlight is not rigorously the same as if it is made with diffused or artificial light which is richer in yellow and poorer in blue. When working with an accuracy greater than 0·1 pH it is better to use a source of constant light, operating under special conditions.

The bactericidal action of mercuric chloride prevents the troublesome activity of micro-organisms present in the sea-water whilst its very small dissociation coefficient ($\alpha = 0{\cdot}013$) is without effect on the pH. If when the samples are collected on board about four drops of a saturated solution of mercuric chloride is added to each 100 c.c., bacterial action is stopped, and furthermore if the sample bottle is completely filled, no air-space being left over the water, evasion or invasion of carbon dioxide is inhibited or at least very greatly restricted.

The determinations of pH in sea-water samples have been carried out by means of the well-known McClendon method (1) in order to obviate the salt error. The buffer solutions were " boric " (18·6 gm. boric acid and

22·5 gm. sodium chloride made up to 1 litre with boiled distilled water) and "borax" (28·67 gm. sodium borate and 19·0 gm. sodium chloride made up to 1 litre with boiled distilled water); the total volume of each standard made by mixing these two buffers in correct proportions was 10 c.c. against 30 c.c. used by McClendon. The indicator was 0·5 c.c. of 0·02% cresol red solution prepared from a 0·2% alcoholic solution. A stock solution of this strength was made in order to avoid the addition of a very little sodium hydroxide necessary for the complete dissolution of cresol red as an 0·6% solution. The test tubes, of Monax resistance glass (12 cm. in height and 1·3 cm. in diameter), were chosen so that, when 10 c.c. of water were added to each, the columns of liquid had approximately the same height. The tubes after washing with distilled water were steamed and finally dried in the stove at 110° C. Ten c.c. of the appropriate buffer mixture, 0·5 c.c. of the indicator and two drops of toluene were placed in each tube, which was closed by a cork coated with paraffin wax. Finally an efficient seal is ensured by a second coat of wax over the cork and top of the tube, and label. The standards remain unchanged and fit for use even after many months (**2**). When not in use the tubes are kept in the dark as a precaution. The standards were at pH =8·40–7·30 with a difference of 0·05 or 0·03.

The addition of two drops of a 2·5% mercuric chloride solution to the sea-water samples in the test tubes does not interfere with the pH, and if these samples containing mercuric chloride are stored in darkness at a temperature of 12-14° C. in test tubes carefully sealed no changes in pH are noticed after some days. In water samples without mercuric chloride a much more rapid fading in the colour is observed. An unpreserved sea-water sample from one mile N.W. of the Eddystone, whose pH on the day of collection was 8·11, gave 8·06 on the following day, and 7·38 after 12 days. A sample of the same water containing mercuric chloride and also stored in darkness at 12–14° C. did not alter its pH for three weeks. Other samples also from the Eddystone, containing mercuric chloride, gave the same value of pH (8·11) for a month, after which the colour changed a little, giving the value 8·07.

The colour of certain samples, preserved and stored in the same way, changed after 5–6 days, but the tubes were found to have been imperfectly sealed. Thus the mercuric chloride stopped the activity of the micro-organisms, but the entry of atmospheric carbon dioxide modified the pH.

If the water samples are taken in ordinary bottles not completely full, to which mercuric chloride is added on collection and the addition of indicator made a few days afterwards, the pH observed is less than on the day on which they were taken. Determinations made on opening the tubes a week later, gave a result 8·05 on the water with mercuric chloride and

TABLE I.

Effect of Mercuric Chloride at 12° C.

St.	Water.	Day of collection.	One day after.	2 days after.	3 days after.	4 days after.	5 days after.	6 days after.	7 days after.	8 days after.	9 days after.	11 days after.	12 days after.	13 days after.	14 days after.	15 days after.	17 days after.	18 days after.	19 days after.	20 days after.	21 days after.	23 days after
L1	alone	8·05	8·04	7·92	7·83	7·58	7·38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
L1	plus merc. chloride	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	8·05	7·97	7·95	7·96	7·95	7·91	7·91	7·91
L2	alone	8·07	8·05	8·03	7 88	7·73	7·68	7·53	7·38	—	—	—	—	—	—	—	—	—	—	—	—	—
L2	plus merc. chloride	8·07	8·07	8·07	8·07	8·07	8·07	8·07	8·07	8 07	8·07	8·07	8·07	8·07	8·07	7·95	7·94	7·91	7·91	7·90	7·90	7·90
L3	alone	8·09	8·09	8·00	7·88	7·78	7·68	7·58	7·58	7·58	7·58	7·56	7·56	7·56	7·56	7·56	7·56	7·50	—	—	—	—
L3	plus merc. chloride	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8·09	8 05	8·05	8·04	8·02	8 01	8·01	8·01
L4	alone	8·10	8·10	8·05	7·93	7·75	7·63	7·63	7·58	7·53	7·48	7·38	—	—	—	—	—	—	—	—	—	—
L4	plus merc. chloride	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·10	8·02	8·02	7·99	7·96	7·96	7·96	7·96
L5	alone	8·11	8·09	8·00	7·93	7·73	7·63	7·55	7·43	—	—	—	—	—	—	—	—	—	—	—	—	—
L5	plus merc. chloride	8·11	8·11	8·11	8·11	8·11	8·11	8·11	8·11	8 11	8·11	8 11	8·11	8·11	8·10	8·11	8·11	8·11	8·11	8·09	8·06	8·06

7·93 on the water without antiseptic. These values are lower than the values found (8·10) on the day when the water was collected.

But if the samples containing mercuric chloride are drawn from bottles completely full the pH determined by adding the indicator some days afterwards is the same as the original value.

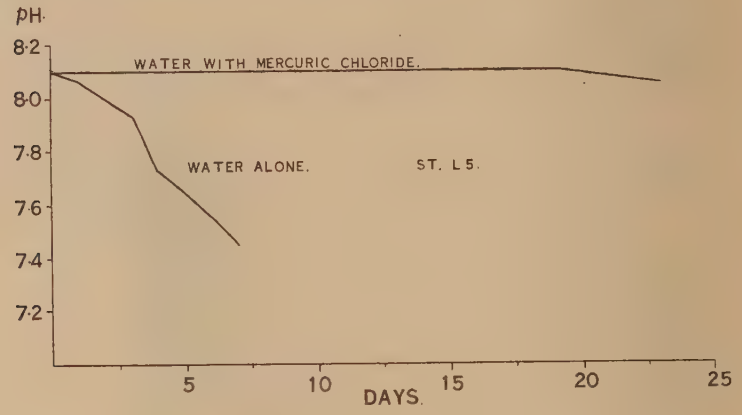

FIG. 1.

The following table contains the results obtained on sea-water samples from the International Hydrographical Stations L1, L2, L3, L4, and L5 (on Feb. 5th) with and without mercuric chloride. These were stored on the day of collection in carefully sealed test tubes and kept in darkness at 12–14° C. The data for Station L5 are shown graphically in Figure 1.

Other preservatives have been tried. Toluene was the best, but as the figures in Table II show, it is much less effective than mercuric chloride.

TABLE II.

EFFECT OF DIFFERENT PRESERVATIVES AT 12° C.

	1st day.	2nd day.	3rd day.	4th day.	6th day.	7th day.	8th day.	9th day.	10th day.
W. alone	8·11	8·11	8·06	7·91	7·78	7·73	7·63	7·53	7·48
W.+ toluene	8·11	8·11	8·10	7·98	7·93	7·93	7·83	7·83	7·83
W.+ mercuric chloride	8·11	8·11	8·11	8·11	8·11	8·11	8·11	8·11	8·11

It is well known that temperature plays an important part in the determination of pH, because heat increases the dissociation of ions and correlatively the pH decreases. The determinations must be made with the water samples at the same temperature as the buffer mixtures.

If the test tubes containing the sea-water samples plus indicator are stored at a temperature higher than 12–14° C. the colour fades more quickly.

TABLE III.

EFFECT OF MERCURIC CHLORIDE AT 33° C.

Stn.	Water.	1st day.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	12th day.
L2	alone	8·07	8·00	7·80	7·43	7·20	—	—	—	—	—	—
L2	plus mercuric chloride	8·07	8·07	8·06	8·02	7·91	7·91	7·91	7·91	7·91	7·83	7·73
L5	alone	8·11	8·06	8·00	7·73	7·23	—	—	—	—	—	—
L5	plus mercuric chloride	8·11	8·11	8·10	8·05	7·88	7·88	7·88	7·88	7·88	7·88	7·88

Figure 2 shows these variations in sea-water from L2 and L5 with and without mercuric chloride kept in a thermostat at 33° C. We noticed that the pH of the sealed samples containing mercuric chloride show no

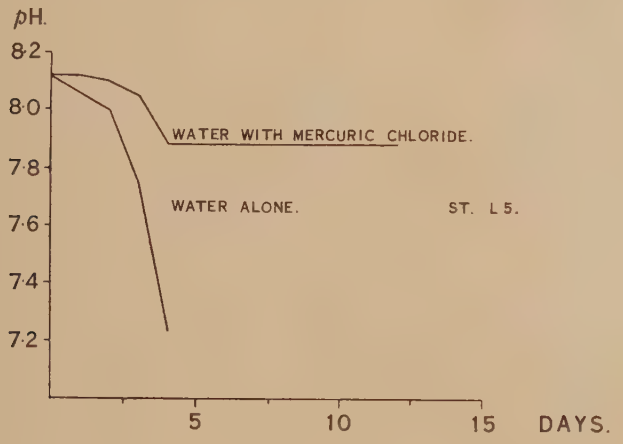

FIG. 2.

change after two days, but that in unpreserved water the decrease is very rapid indeed, somewhat naturally even more rapid that at the lower temperature.

The buffer mixtures did not change at 33° C. as judged by subsequent comparison with buffers kept at 12° C.

The erratic decreases are owing to different causes. According to Legendre proteins can influence the indicators, perhaps partially absorbing them, and although the sea-water contains these nitrogenous substances in very small amount, different proportions of them may produce a variable fading in the colour.

It was stated above that to every test tube containing McClendon mixtures two drops of toluene were added, to preserve them ; but it is possible to work under the same conditions by adding two drops of a 2·5% mercuric chloride solution in place of toluene, making a correction in the value of pH. As is well known mercuric chloride gives a precipitate of basic salt with sodium borate, but in the presence of boric acid there is no precipitation since a complex is formed. The pH is therefore slightly changed. Buffer mixtures were prepared in this way and their values were 0·03 less than in the original mixtures with toluene.

The author desires to thank Mr. H. W. Harvey and Dr. L. H. N. Cooper of this Laboratory for their interest in this work.

SUMMARY.

1. It is not always feasible to determine pH on board ship, owing to bad light and other conditions, although it may be possible to collect water samples.

2. By preserving the water samples on collection with mercuric chloride solution (four drops per 100 c.c. of water) and by taking care to leave only a small air-space in the bottles it is possible to keep the water practically unchanged for many days.

3. Test tubes containing such water (10 c.c.) plus 0·02% cresol red (0·5 c.c.) have been kept unchanged for three weeks at 12–14° C. and for two days at 33° C., whereas unpreserved water showed a drop in pH of 0·05 unit in one day at 12° C.

4. McClendon buffer solutions may also be preserved by mercuric chloride, but in this case a small correction must be applied since complex formation leads to a removal of effective buffer salts from the solution and a small decrease in pH.

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On Abnormal Conditions of the Gills in *Mytilus edulis*. Part II. Structural Abnormalities, with a Note on the Method of Division of the Mantle Cavity in Normal Individuals.

By

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Amy, Lady Tate, Scholar of Bedford College.

With 27 Figures in the Text.

CONTENTS.

PAGE

INTRODUCTION 489

DESCRIPTION OF STRUCTURAL ABNORMALITIES OF THE GILLS 490

(*a*) Folding over of the free ventral edge of the gill, with concrescence . . 490

(*b*) Fusion of the gill filaments side by side 500

(*c*) Enlargement of the gill filaments 509

(*d*) Concrescence of the two gills of one side 515

GENERAL CONDITION OF MYTILUS FROM THE VARIOUS LOCALITIES INVESTIGATED 516

DISCUSSION 523

SUMMARY 531

METHOD OF DIVISION OF THE MANTLE CAVITY IN NORMAL INDIVIDUALS . . 533

INTRODUCTION.

IN Part I of this paper (2) the presence of secondary grooves and folds on the gills of *Mytilus edulis* especially was described, together with the occurrence of permanent natural reversal of the frontal cilia on the gill filaments composing the majority of these grooves and folds. The present paper is concerned with certain, chiefly structural, abnormalities of the gills, namely: (*a*) folding over of the free ventral edge of the gill with concrescence, (*b*) fusion of the gill filaments side by side, (*c*) enlargement of the gill filaments and (*d*) concrescence of the two gills of one side. The mussels in which the first two of these conditions were observed, were almost entirely from various parts of the Fal Estuary, the average percentage with abnormal gills being 31·8% among 1398* recorded from October 28th to November 25th, 1927, and 44·4% among 162 examined in March, 1930. In no other locality from which mussels have been obtained, was anything approaching these conditions seen, though a very

* Given in error as 1488 in (2) Part I, p. 919.

occasional specimen might have abnormal gills. Batches of mussels have been examined from the Estuaries of the Hamoaze, mostly from near Weir Point (1291 between October 8th, 1927, and February 17th, 1928); the Estuary of the Yealm (296 between October 10th, 1927, and August 3rd, 1928); the Estuary at Teignmouth (9262 between December 6th, 1927, and February 26th, 1929); the Estuary of the Camel, near St. Issey Cliff, Padstow (10,866 between November 8th, 1927, and August 9th, 1929); and from the Promenade Pier, Plymouth (340 December, 1927, and September, 1928).

The widespread abnormal conditions of the gills of the Fal Estuary mussels would seem to be correlated, most probably, with some factor or combination of factors in the environment and not to be due to injury, though several cases of accessory palps, divided palps, and accessory feet possibly had a traumatic origin. The percentages of pea-crabs (*Pinnotheres pisum*) in the Fal Estuary mussels of 1927 were so low (see Table I, p. 517) that their presence could have no relation to the abnormal conditions of the gills, and in the 1930 sample from that locality, mussels containing pea-crabs, of a size likely to cause injury, have been omitted from the total on which the percentage of 44·4 is based. In a certain few cases—about three in March, 1930—in which the gills were exceedingly narrow, or nearly missing, for a short distance, the injury could be almost certainly traced to an old boring by the whelk-tingles, Murex or Purpura. Apart from these few cases, it is suggested that the abnormal conditions of the gills of the Fal Estuary mussels are correlated with some peculiar factor or combination of factors in the environment, though it is by no means clear why mussels from the estuaries of the Hamoaze, Yealm, Teign, and Camel are so little affected.

A consideration of abnormality in the gills of Mytilus is of some importance in regard to the problem of purification of this mollusc for consumption (**12**).

DESCRIPTION OF STRUCTURAL ABNORMALITIES OF THE GILLS.

(*a*) Folding over of the Free Ventral Edge of the Gill, with Concrescence.

This abnormal condition of the gills was restricted to the Fal Estuary mussels, except for its occurrence, in a much modified degree, in a few mussels from other localities, which were inhabited, in practically all instances, by pea-crabs. The deep and regular folding over of all four gills,* as shown in Figure 1, has been observed to occur only in the Fal

* As in Part I (**2**), for convenience in description the two demibranchs on each side of the body are considered as two gills.

mussels, a number of which had the gills permanently folded over lengthwise, the fold in some cases extending up to two-thirds of the length of the gill. The extreme anterior and posterior ends of the gills were very rarely involved in the fold. It is peculiar that with few exceptions (one exception only noticed) the gills were folded away from each other, the inner gill of either side being folded inwards, and the outer gill outwards (cf. the direction of upward folding of the ventral tips of the descending filaments during development). The filaments in the folded over portion tend to become fused or concresced in varying degree with those beneath them ;

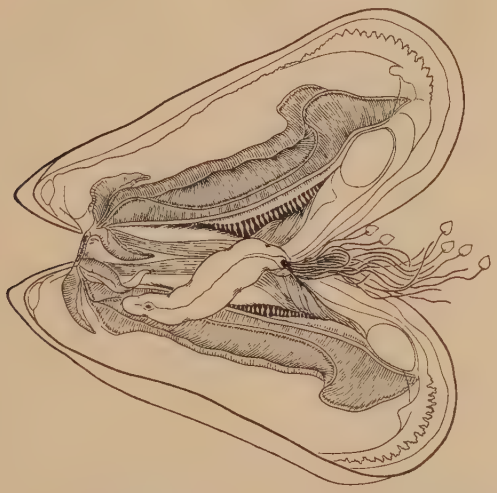

Fig. 1.—Sketch from life of a mussel from the Fal Estuary, October 28th, 1927, showing folding over of the four gills. The folded portion of the left inner gill alone was entirely fused ; that of the other three gills could be raised almost to the bend. Natural size.

various stages in the process were observed. In the very early stages the entire fold can be raised, though when placed in water the gill will not straighten out. Fusion first occurs near the bend of the fold and the major part can be raised ; in later stages the folded over portion has become entirely fused with the lamella beneath. In the mussel sketched in Figure 1 the fold of the left inner gill alone was entirely fused, those of the other three gills could be raised almost to the bend.

The appearance in surface view of a small part of a gill in which the fold had become completely fused, is shown in some detail in Figure 2 (p. 492). The main food groove now runs across the surface of the lamella (at G), and just dorsal to it there is a narrow zone in which fusion of the filaments side by side, and considerable irregular proliferation of the frontal surfaces

of the filaments has occurred. Fusion of the filaments has also taken place along a narrow region ventral to the food groove, as well as near the bend of the fold, at what is now the free edge of the gill. The occurrence of fusion of the filaments laterally, added to the fact that the folding is

FIG. 2.—Surface view of a small area of a gill of which the folded portion had entirely fused, the main ventral food groove (G) now running across the surface of the lamella. The fusion of the gill filaments side by side is shown, and the irregular growth of the frontal epithelium in a narrow region dorsal to the fused food groove. The ventral and folded edge of the gill is at the top of the figure. From a mussel from East Bank, Fal Estuary, November 23, 1927. From preserved material. × ca. $24\frac{1}{3}$.

usually slightly oblique—a filament generally being folded over on to one posterior to it in the series—makes it difficult to separate a single filament, or even a few filaments, to examine in side view.

Figure 3 shows in side view small groups of filaments from gills

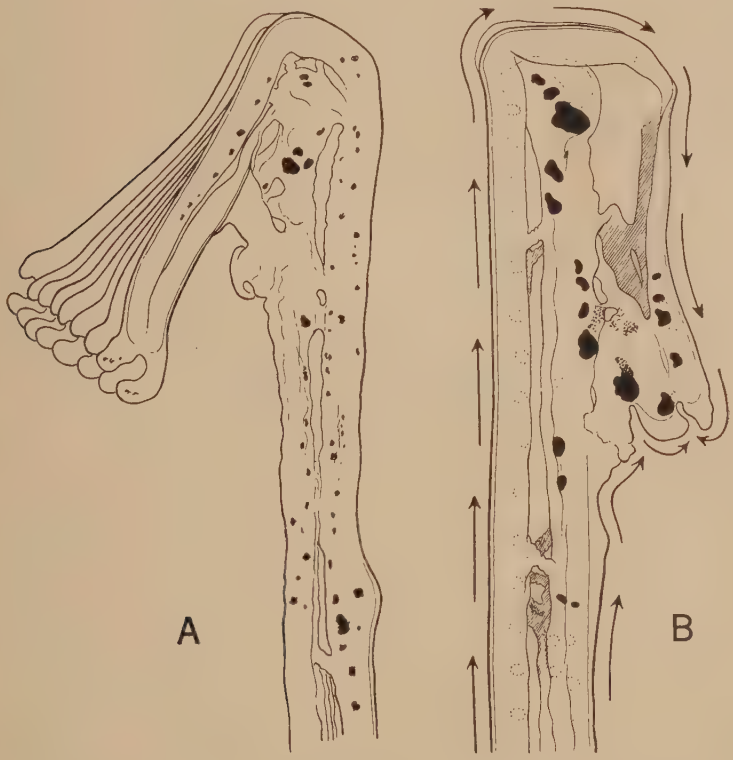

FIG. 3.

A. Side view of a small group of filaments from a gill of Mytilus in which the folded over portion had fused at the bend only. Considerable fusion of filaments side by side had occurred, and the surface sketched showed no ciliated discs, while lateral and latero-frontal cilia were absent for the most part, indicating presumably that fusion with the next filament in the series had already begun. The accumulation of brown pigment in masses is shown. From a mussel from East Bank, Fal Estuary. November, 1927.

B. Side view of a small group of filaments from the same gill as that of Fig. 2, showing complete fusion of the folded over portion of the gill with the ascending lamella. The two inner arms of the fold have apparently fused and are undergoing degeneration: masses of brown pigment are indicated. There is some fusion of the ascending filaments laterally, dorsal to the food groove, with slight enlargement of the frontal surfaces of the filaments. The arrows, showing the direction of the ciliary current on the frontal surface, have been added from a living gill, which showed a similar fold and fusion.

A–B, from preserved material. $\times 18\frac{1}{4}$.

with different degrees of fusion of the folds. Figure 3, A, was from a gill where the fold had only fused at the bend. There was much fusion of the filaments side by side dorsal to the fold, and the lateral surface sketched showed no ciliated discs, and, for the most part, lateral and latero-frontal cilia were absent, indicating that fusion with the neighbouring filament had begun. As is well shown by the example in Figure 3, B, fusion and degeneration of the two inner arms of the fold take place, and pigment—no doubt liberated from degenerating cells—is generally found collected into granular masses of considerable size. Transverse sections show varying degrees of fusion in different parts, and at different levels of the same fold. In some places four lamellæ are distinct, in others three, the two inner ones having fused. In one fold sectioned the middle one of the three lamellæ was seen to be formed by the fusion of two lamellæ, that is the two inner arms of the fold, as clearly shown by the presence of two series of chitinous supports, mostly somewhat contorted (see Fig. 4, A, p. 495). In another fold sectioned, however, though four lamellæ were present in places, yet where only three were present, the chitinous supports could not be clearly distinguishable into two sets ; this perhaps may be due to the obliquity of the folding. In one example sectioned, where the apparent fold was only about 1·6 mm. deep, the middle lamella of the three present was clearly single—as shown by the distinct single set of poorly developed chitinous supports—and was the ascending lamella (Fig. 4, B). Complete fusion side by side of all the filaments in the middle lamella had not taken place, a few small ciliated spaces remaining. This case would not seem to be explicable by folding alone, but possibly also by differential growth.

Some gills which appeared in surface view to have undergone folding, showed when the filaments were examined in side view, an appearance as in Figure 5 (p. 496). The ascending filaments were only about a third the length of the descending, and in consequence the descending filaments appear to have been pulled over. It would seem that the shortness of the ascending lamella was due to injury to, or puckering of, the filaments composing it. The gills of the mussel in which this condition was noted, had, however, very few interlamellar connexions in the abnormal portion ; possibly they had snapped at some time, for there were small masses of subfilamentar tissue.

In gills where folding with concrescence has occurred, the ascending lamella is sometimes considerably shorter, dorso-ventrally, than the descending one; the question therefore arises whether gills in such a condition will be able to effect a ciliary junction with the mantle and the visceral mass (see Orton, **33**, pp. 460, 462 ; Dodgson, **12**, pp. 168, 171, and the present paper, p. 533). If they are unable to do so there would be imperfect division of the pallial cavity into supra- and infra-branchial

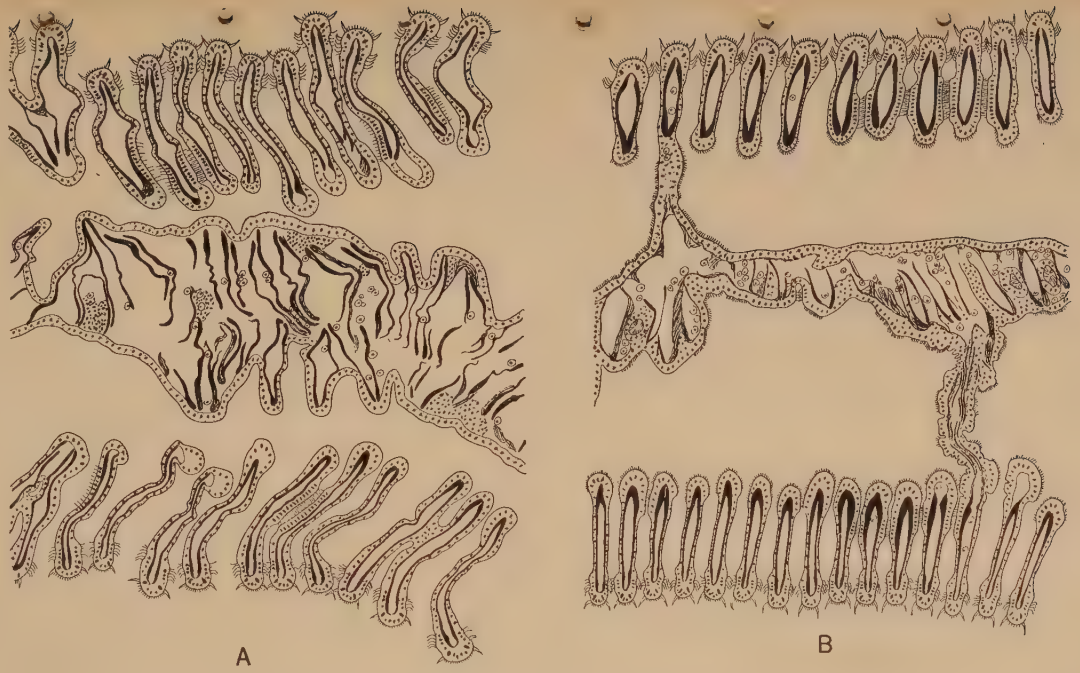

FIG. 4.

A. *M. edulis*. Transverse section through region of a fold—such as shown in Fig. 2—showing three lamellæ, the middle one formed by the fusion of the two inner arms of the fold, the two sets of chitinous supports being clearly discernible in places. The material, fixed in formalin, was badly preserved, but sufficiently to show the chitinous supports. The distribution of the nuclei and cilia is represented diagrammatically: the abfrontal cilia and the numerous gland cells, present especially in the epithelium of the middle lamella, have been omitted. Iron hæmatoxylin and acid fuchsin. $\times 93\frac{1}{3}$.

B. Transverse section through folded region of a gill (fold only ca. 1·6 mm. deep) in which the middle lamella, as shown by the single series of chitinous supports, is single. Complete fusion laterally of all the filaments in the middle lamella had not occurred, a few small ciliated spaces remaining. The difference in width—from frontal to abfrontal surface—of the filaments in the two outer lamellæ is due to the section being slightly oblique. The distribution of the nuclei and cilia is represented diagrammatically. Bouin's fixative; Mann's methyl-blue-eosin. $\times 93\frac{1}{3}$.

chambers; the exhalent current would, in all probability, cease or diminish in strength, and the feeding mechanism would be most probably deranged (see Dodgson, **12**, p. 171). In some mussels with abnormally short ascending lamellæ it has been noticed that such short lamellæ seem

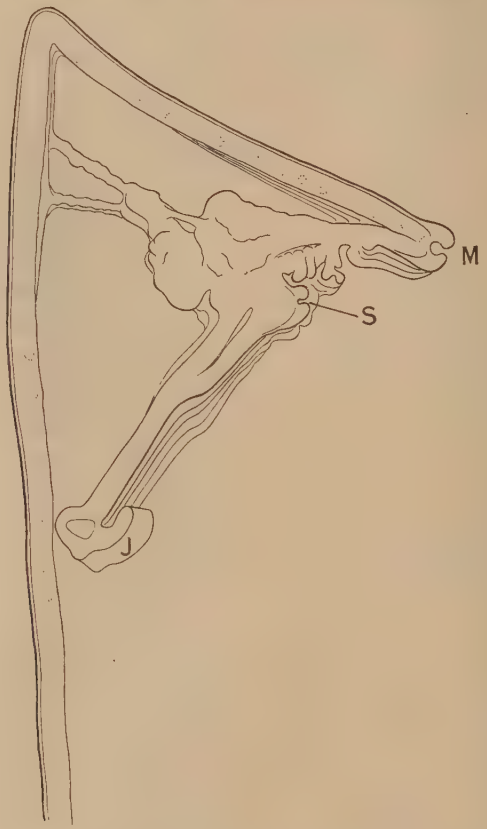

Fig. 5.—Lateral view of a small group of filaments from the gill of a mussel from East Bank, Fal Estuary, November 23, 1927, which showed an apparent fold in surface view. M, main ventral food groove; S, secondary food groove; J, junction area of interlocking cilia on outer surface of dorsal food groove. From preserved material. × ca. 12⅛.

to be turned out almost at right angles, whence it is possible that the dorsal free edge of the gill, by pulling the gill over towards the side of junction, is able to touch the mantle or the visceral mass. In a few mussels with gills in this condition, a well-defined ridge in the mantle has been noticed (as in Fig. 6, B and C, p. 497), which may help to enable the

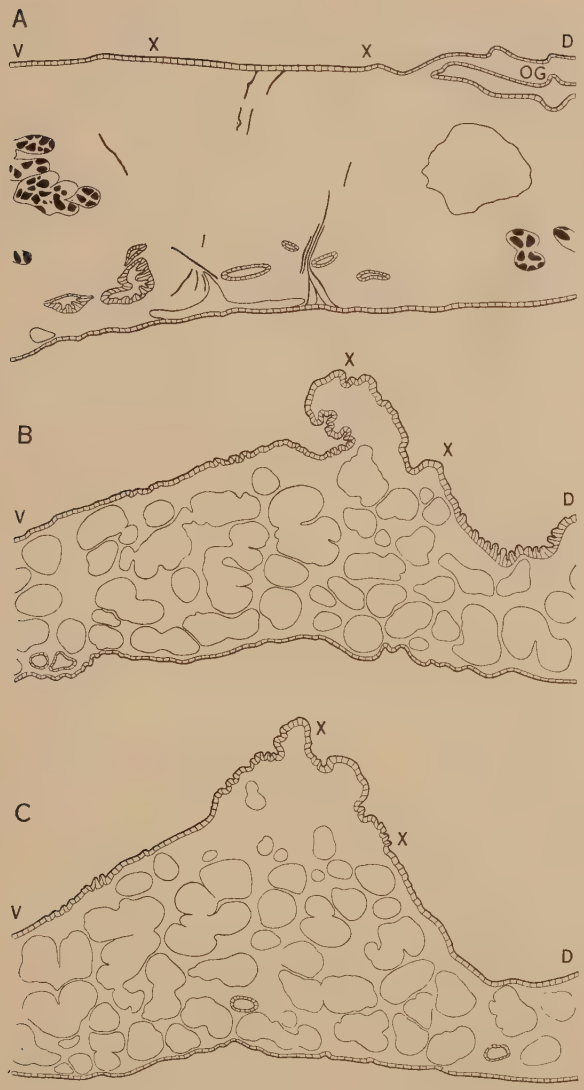

FIG. 6.

A. Transverse section of mantle of normal Mytilus, passing through the zone of interlocking cilia, X to X. OG, plicate organ; D and V, Dorsal and Ventral.

B and C. Transverse sections through different regions of a ridge present in mantle of a Mytilus, in which the gills of that side were extremely short, dorso-ventrally. X–X marks the position of the zone of interlocking cilia on the dorsal slope of the ridge.

D and V, Dorsal and Ventral.

A–C. × ca. 27½.

outer gills to effect a ciliary junction with the mantle. The interlocking cilia (see p. 533) are present on the dorsalward slope of the ridge. Such a ridge tends to become lower some time after the mussel is opened, and also on preservation, perhaps indicating that it is partly due to turgescence.

A marked ridge in the mantle (Fig. 6, B and C) is also generally found in mussels with a gill, or gills, almost entirely absent for a short distance, as may occur through injury, in some instances by whelk-tingles. It would appear to be an effort of the mantle to effect a ciliary junction with the rudimentary or missing part of the gill. A ridge in one instance was noticed to be strongly pigmented.

Gills in which folding has taken place, occasionally have short secondary food grooves along the bend, at what is now the free edge of the gill. It is perhaps only when the filaments are split or injured at the bend that a new food groove arises in this position.

Mussels with folded gills were often in poor condition : the folding, involving considerable reduction of the gill surface and pumping power, as well as frequently disorganising the main food grooves, must necessarily reduce the quantity of food passing to the mouth. In cases, however, where the gill is folded over neatly, as in Figure 1 (p. 491), beyond causing a certain reduction in the area of the catching surface, the food paths would not seem to be greatly disorganised, as particles on the descending filaments pass round the bend and into the main food groove—though it now runs across the surface of the gill—and are carried along it towards the mouth.

Figure 7 (p. 499) shows to what an extent the normal food currents of a gill may be disorganised by irregular folding with concrescence. All four gills of this mussel were in a similar condition. When the gills were supplied with carmine particles it was evident that there was no continuous ciliary current at, or near, the ventral free edge of the gill, or in any position on the gill. Particles collected in small masses, either because of the meeting of ciliary currents, or because of the abrupt termination of short irregular food grooves. No doubt when these reached a certain size they would spill over, and coming under the influence of other currents, might in some cases travel a little further towards the mouth. As all the gills were short there was no likelihood that particles dropping from one or other of the left or right gills would be caught up by the other one of that side. In spite of the apparent impossibility of food in any quantity being able to reach the mouth, the mussel in this particular instance was in fair condition. This perhaps may be regarded as an indication that the changes in gill structure were rapid.

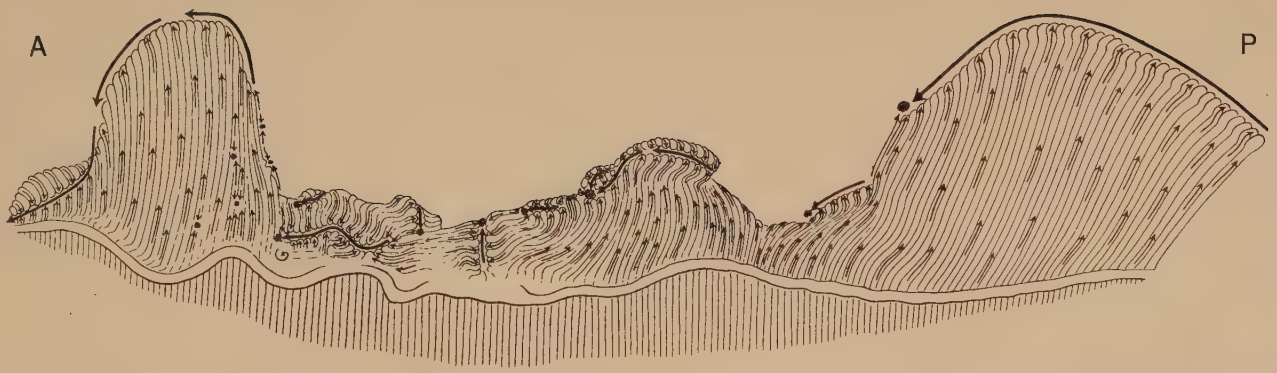

FIG. 7.—Sketch of living gill from a mussel from North Bank, Fal Estuary, 1930, showing disorganisation of food currents by what is, presumably, irregular folding of the gill with fusion. All four gills of this mussel were in a similar state. The direction of the currents along food grooves is indicated by heavy arrows; the direction of the currents caused by the frontal cilia on the filaments by fine arrows. The currents were investigated only by means of powdered carmine on the surface of the gill. A certain amount of reversal of ciliary current appeared to have occurred as indicated. Towards the anterior end of the gill reversal occurred on filaments without secondary grooves: some fusion of filaments laterally had taken place in this region. In two places the dorsal food groove on the ascending lamella was missing. The ascending lamella was considerably shorter than the descending lamella. A, anterior; P, posterior end of gill. The outline of the gill was drawn by camera lucida, but the details were filled in freehand. × ca. $4\frac{1}{2}$.

(*b*) Fusion of the Gill Filaments side by side.

Fusion and crumpling of the filaments, which was greatly developed among the mussels from the Fal Estuary, was very rarely found among mussels—other than those infected with Pinnotheres—from other localities: exceedingly few examples were detected among those from the Estuaries of the Hamoaze and from the Teign Estuary.

This condition varies greatly in extent from the fusion of two or more filaments side by side, to extensive areas of fusion scattered irregularly over the greater part of the lamella.

Some fusion is generally found where slight crumpling or puckering of the gill occurs, as in Figure 8, A (p. 501), and is probably due to crowding of the filaments. In the case of crumpling, the filaments are not only crowded together, but there is also a tendency for them to be forced on to their sides, and, as the lateral faces are of greater width than the frontal, this increases the crowding. In some instances small areas of fusion may be accompanied by a tiny food groove as in Figure 8, B.

Slight fusion of filaments has also been noticed, in some individuals, near the dorsal food groove on the ascending lamella (Fig. 8, C), and is again probably due to the same direct cause, namely crowding, occasioned by slight shortening, antero-posteriorly, of the dorsal free edge of the gill. In the example sketched, seven filaments are fused, but at the food groove the composite filament is not noticeably wider than the normal filaments (see also Rice, **41**, pp. 74, 78).

In some few cases the dorsal food groove on the ascending lamella was seen to be noticeably shortened antero-posteriorly—due apparently in the case sectioned to the presence of cysts*—so that the ascending lamella was thrown into folds parallel to the long axes of the filaments, the folds decreasing in depth ventrally; while the descending lamella was hardly affected, the folds being exceedingly slight and not sufficient to cause fusion of the filaments. Transverse sections showed much fusion of the filaments composing the deep folds of the ascending lamella (Fig. 9, p. 502): the fusion was, however, almost entirely restricted to the abfrontal ends of the filaments and would have been practically invisible in surface view.

Fusion, according to Rice, does not normally occur in filamentous gills, even where folding is extreme, as in Pecten, and he says that its absence "may be easily explained on the ground of the looser structure of the gill (as compared with the folded lamellar gills of certain Eulamellibranchs†) and the possibility of a displacement of the filaments, with consequent

* The cyst sectioned was lined with ciliated epithelium: the contained body had a roughly concentric structure, but was evidently degenerating and could not be identified.

† The interpolation is mine.

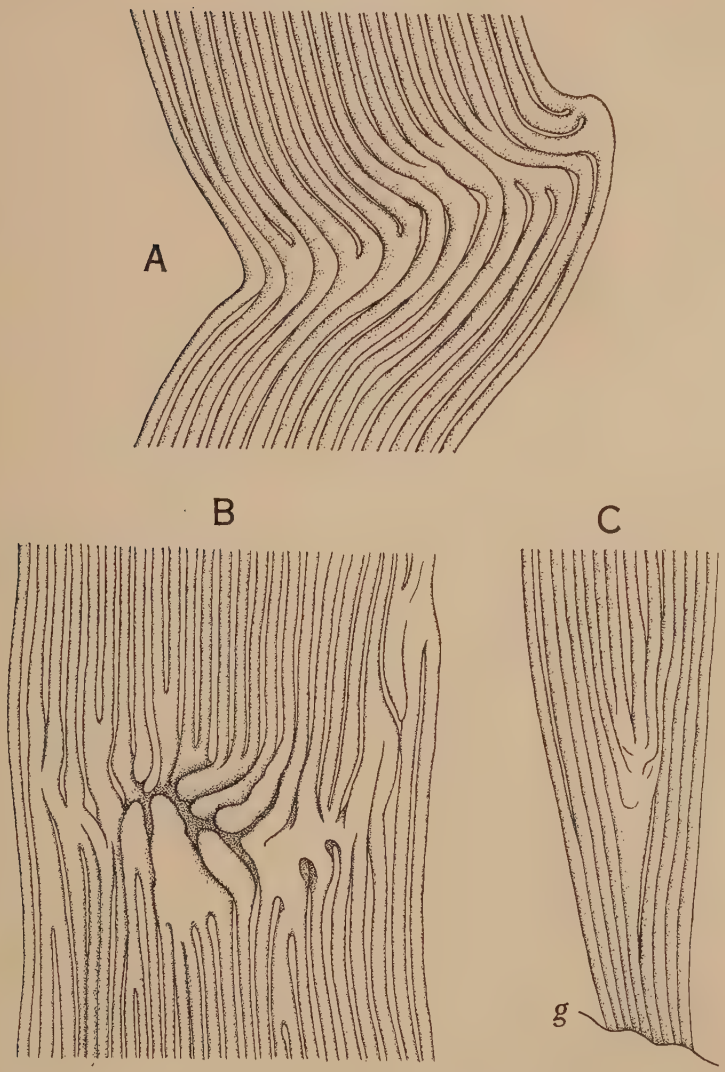

FIG. 8.—Sketches showing fusion of gill filaments on the gills of a mussel from Trelissick Reach, Fal River, November 1, 1927. From preserved material. × ca. 41½.

A. Fusion of gill filaments where crowding had occurred, owing to the filaments being bent out of their true direction. This is an enlargement of a small area, just posterior to the secondary gill or fold, on the right inner gill sketched in Fig. 1, p. 920, Part I (2).

B. Fusion of gill filaments, together with a tiny secondary food groove. From right outer gill. (Anterior is on the left.)

C. Fusion of seven filaments near the dorsal food groove on the ascending lamella, right inner gill. g, ventral edge of dorsal food groove.

relief of pressure " (**41**, p. 78). Its presence in Mytilus gills, which have been thrown into folds, is therefore of interest.

A section such as that of Figure 9 has a superficial resemblance to those of *Avicula argentea* (**43**, Fig. 16, p. 212) and *Margaritifera vulgaris* (**24**, Figs. 7, 12, Pl. 27), but in these two forms fusion of the actual filaments

FIG. 9.—Transverse section through dorso-ventral folds in an ascending lamella, near the dorsal food groove, of a mussel from North Bank, Fal Estuary, 1930. The distribution of the cilia and nuclei is represented diagrammatically. The extensive area of ciliation on the abfrontal surface of the lamella, is, however, noteworthy. Bouin's fixative ; Mann's methyl-blue-eosin. × ca. 93⅓.

does not occur ; there is a tendency for the filaments to form interlamellar extensions, particularly in the region of the ciliated discs, which by their fusion form interfilamentar junctions ; this is clearly seen from Herdman's Figure 6, Plate 27 (**24**).

The foregoing examples of fusion would appear to be due to crowding,

consequent on the puckering and folding of the lamellæ—whatever may be the cause of the latter—but it is more difficult to suggest a cause for the extensive areas of fusion involving irregularly the greater part of a lamella.

A crumpled or puckered appearance of the entire surface of the lamellæ of some gills has been noticed—sometimes restricted to one lamella of a

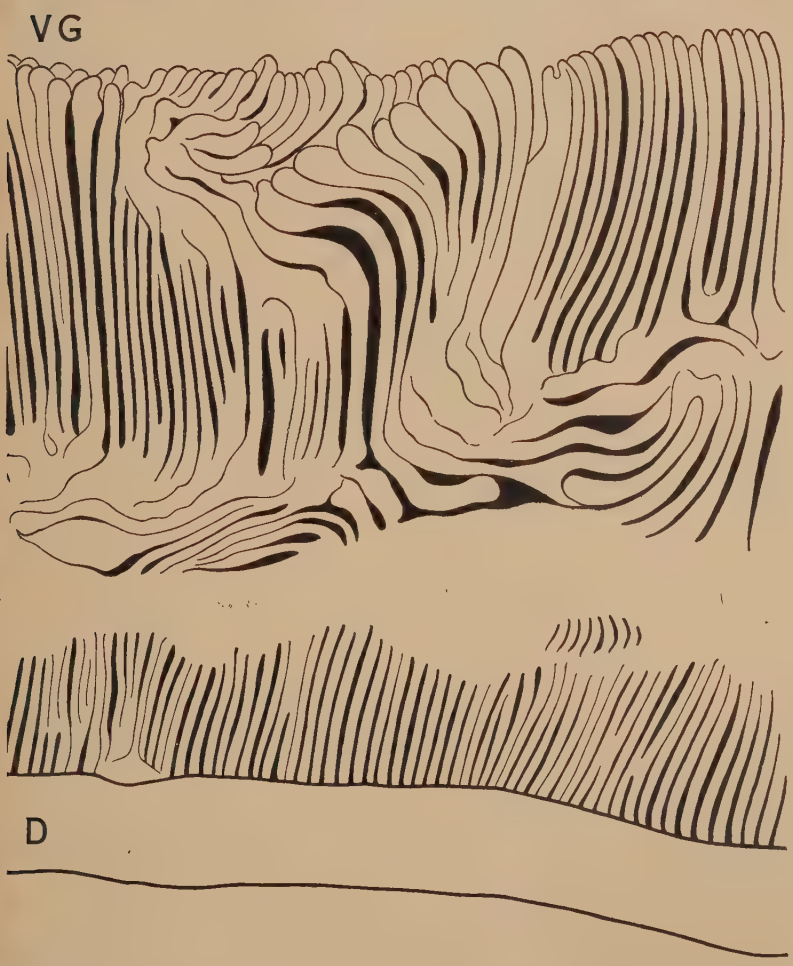

Fig. 10.—Sketch of part of ascending lamella of a gill, showing contortion and fusion of the filaments; in some places the filaments are almost at right angles to their normal direction. The ascending lamella was considerably shorter, dorso-ventrally, than the descending lamella. VG, ventral edge of gill; D, dorsal food groove. From a mussel from Mylor Bank, Fal Estuary, November 22, 1927; preserved material. × ca. 24⅓.

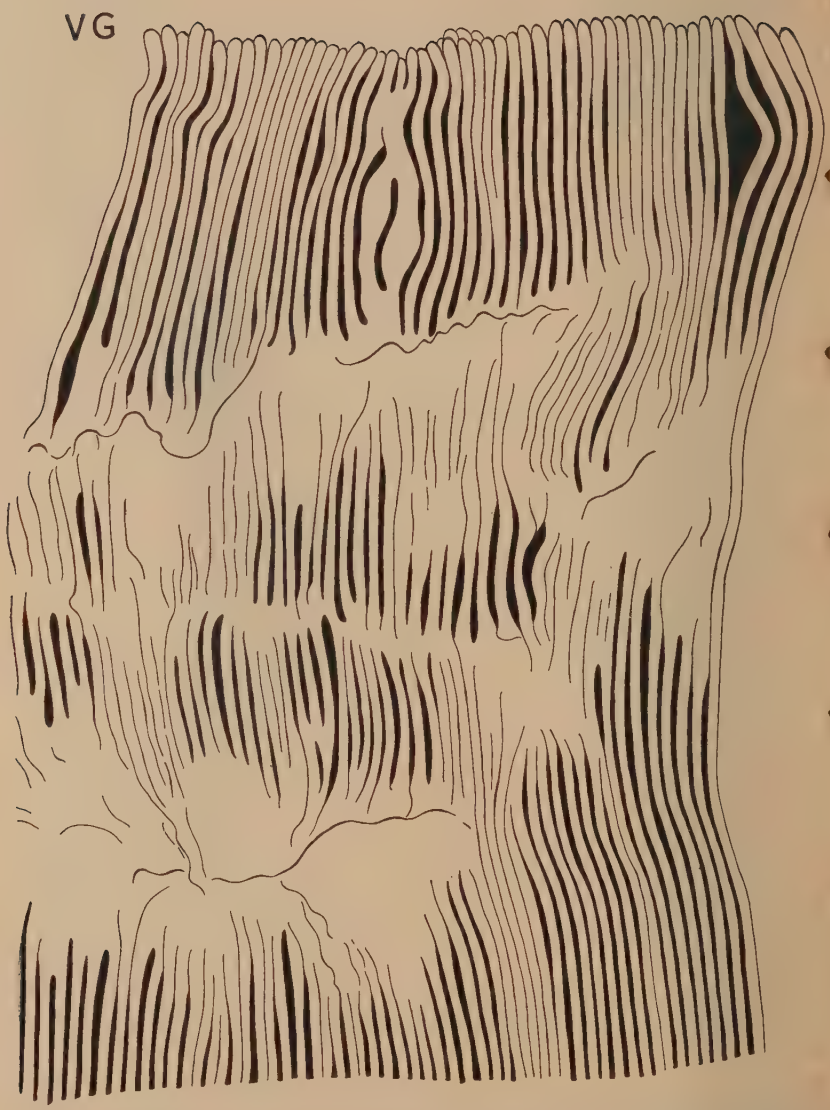

FIG. 11.—Sketch of part of ascending lamella of a gill, showing considerable areas of fusion of the filaments. The living gill showed much brown pigment, collected in masses. VG, ventral edge of gill. From a mussel from near the junction of the Tamar and Tavy (Weir Point), November 3, 1927: preserved material. × ca. 24⅓.

gill and that most usually the ascending lamella—which remains when the gill is placed in water, and this condition may possibly be the forerunner of cases of extensive fusion, where the filaments, when distinguishable, appear to have undergone a kind of puckering, some running almost at right-angles to their normal direction (Fig. 10, p. 503).

In other instances, however, the fused areas have an approximately flat surface (Fig. 11, p. 504), which has the appearance of being due to the fusion side by side of undisturbed filaments, and it is questionable whether this may not have occurred in certain circumstances, consequent on the

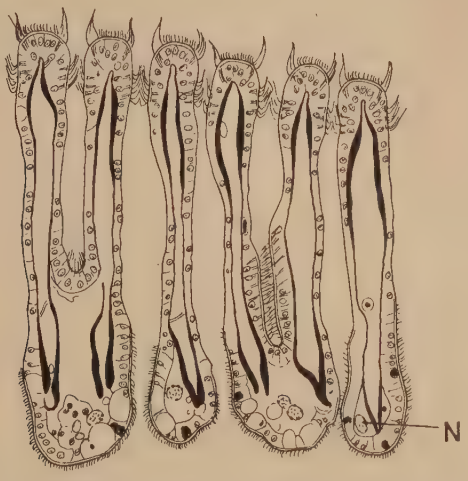

Fig. 12.—Transverse section, single gill lamella, showing fusion of the interlamellar (abfrontal) ends of filaments. N, ? nerve. Bouin's fixative; Mann's methyl-blue-eosin. × 210.

possible swelling of filaments. It is, however, perhaps only a further stage in the process of fusion, masking the irregular surface which follows puckering. The irregular disposition of the chitinous supports, as seen in sections, suggests this possibility, though they have doubtless undergone a certain amount of distortion during embedding and sectioning.

Probable stages in the process of fusion are given in Figures 12 ; 13, A and B ; and 14. Figures 12 and 13, A and B (p. 506), are from a series from one gill of a mussel from the Fal Estuary, 1930 ; Figure 13, A and B, from different levels of the same lamella, and Figure 12 from the opposite lamella. Figure 14 (p. 508) is a section through part of the same lamella as that sketched in surface view in Figure 11 (p. 504), and was from a mussel from near Weir Point (Tamar Estuary). While the ascending lamella showed extensive areas of fusion, the descending one was almost

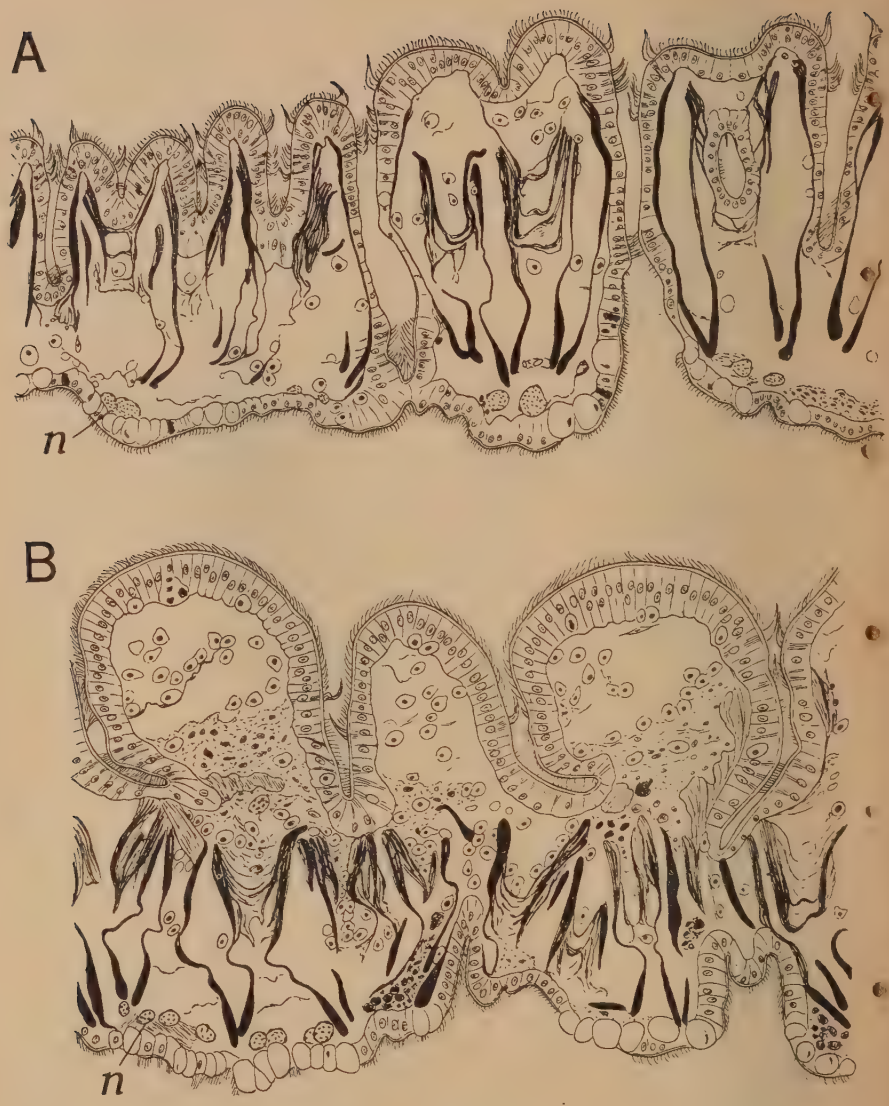

FIG. 13.

A. Transverse section of part of the opposite lamella to that of Fig. 12, showing a further stage in fusion. Towards the centre of the figure three filaments have fused—as shown by the three sets of chitinous supports—though the frontal surface has the appearance of two fused filaments. Between the three sets of chitinous supports are strands or loops of pale staining chitin. The abfrontal epithelium is continuously ciliated, except for the numerous gland cells, which are swollen and pale staining, or darkly staining and granular. n, ? nerve. Bouin's fixative; Mann's methyl-blue-eosin. × 210.

B. Transverse section, single lamella, where fusion is pronounced. Not only has fusion occurred, but also a certain amount of abnormal proliferation of the frontal surface of the lamella in the form of broad ridges and bosses. Ciliated discs persist in some of the furrows, and also latero-frontal and in some instances the lateral cilia. The frontal ends of the chitinous supports are abnormally developed, the additional chitin being pale staining. Gland cells are numerous in the abfrontal epithelium. n, ? nerve. Bouin's fixative; iron hæmatoxylin and acid fuchsin. × 210.

normal, very little fusion occurring: this restriction of fusion to one lamella while the opposite one is almost normal is a rather common feature.

The ridges and bosses caused by irregular proliferation of the frontal epithelium, as in Figure 13, B, may possibly in turn fuse to give a surface resembling that of Figure 14. This is perhaps indicated by the two enclosed spaces—one showing the remains of ciliated discs—and the deep furrow shown in that figure.

While the fused epithelial surfaces doubtless degenerate and are removed by the action of phagocytes, the chitinous supports of the filaments would appear to be very resistant. They are, therefore, in the majority of cases, clear and reliable indicators of the number of filaments which have fused to form a certain stretch of fused lamella, and would seem to offer definite evidence that it is fusion which has occurred.

It may be noted in Figure 13, A, that the central portion of the section, which from the frontal surface would appear to have been formed by the fusion of two filaments, is in reality formed by the fusion of three, as shown by the chitinous supports. In this part of the section the gradual retreat of the spaces, or the smoothing out of the furrows, separating the three filaments, is clearly shown by the successive positions of the strands or loops of pale staining chitin.

The great depth of the cells of the frontal epithelium as shown in Figure 13, A and B, especially in Figure 13, B, may be noted; it will be referred to again under the following section.

Areas of fusion are often visible at a glance owing to their heavy pigmentation, the pigment frequently occurring in dark brown or orange masses of considerable size. Mytilus gills are normally somewhat pigmented, especially along the four main food grooves at the free ventral edges of the gills. The colour is generally yellow or pale orange, though in young normal mussels from Padstow the gills were tinted, more or less entirely, a bluish purple. Following fusion, and the degeneration of a certain amount of tissue, the pigment granules are doubtless liberated and collect in masses (cf. the pigment, which following the transformation of tissue previous to regeneration in *Tubularia mesembryanthemum*, is found lying in a ball within the digestive tract of the newly formed hydranth (**30**, pp. 59, 268).

The ciliation of some areas of fusion was investigated by means of powdered carmine. The ciliary current mostly goes ventrally towards the free edge of the gill, though not always directly ventrally; it may pass somewhat diagonally across the area of fusion. In other cases investigated there were small areas of reversal—judged entirely by movement of carmine particles—and sometimes a swirl of current. Such variations

FIG. 14.—Transverse section, ascending lamella, showing extensive fusion of the filaments (17 filaments in the part sketched). This is from the lamella, part of which is shown in surface view in Fig. 11. The frontal surface is approximately flat except for a deep furrow towards the right side of the section: in the furrow latero-frontal and lateral cilia are still discernible. Two enclosed spaces, due to incomplete fusion of the filaments, are present; ciliated discs persist in one of these. The frontal half of the chitinous supports have become enlarged by the addition of layers of pale staining chitin. A certain number of gland cells are present among the ciliated cells of the abfrontal epithelium. Small masses of granular pigment occur. N, ? nerve. Bouin's fixative; iron hæmatoxylin and acid fuchsin. × 210.

may possibly depend on whether the fused filaments were straight, askew, or crumpled.

(*c*) Enlargement of the Gill Filaments.

The enlargement of the gill filaments was a rare occurrence: the majority of such examples—five—came from the estuary at Teignmouth, and one from the Fal Estuary in 1930. (Enlargement of an occasional filament or tiny group of filaments may be found on a gill beneath a pea-crab.) The most striking example was a mussel from Teignmouth

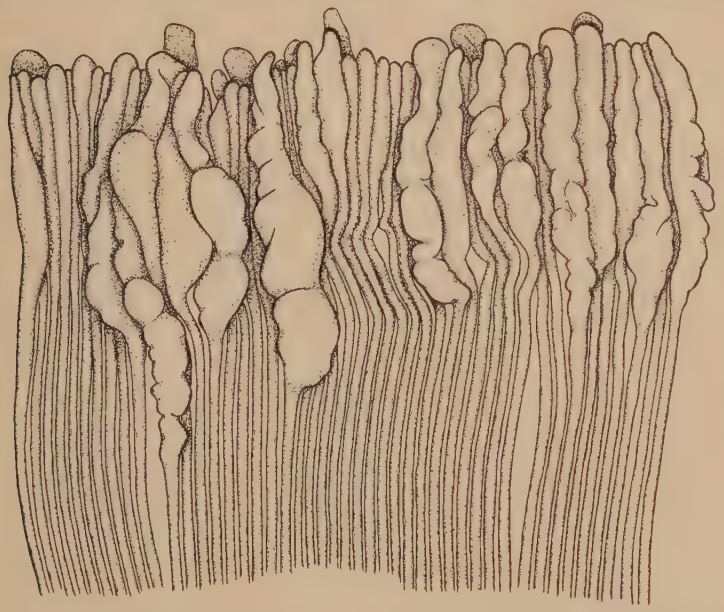

Fig. 15.—Surface view of a small region of an ascending lamella to show enlargement of the filaments in a zone 2·0 to 3·0 mm. wide at the ventral edge of the gill. The main food groove has an irregular appearance owing to the extension ventrally of some of the filaments. From a mussel from Teignmouth, August 21, 1928; preserved material. × ca. 24⅓.

(August 21st, 1928) which showed enlargement of the filaments, generally in small groups, in a zone about 2·0 to 3·0 mm. wide along both sides (descending and ascending lamellæ) of the free ventral edges of all four gills. The enlarged filaments had an irregularly swollen appearance, being pitted in some places with small pockets, and tended to overlie laterally the normal filaments, where these occurred (Fig. 15). While the width across the frontal surface of a normal filament is about 0·05 mm., in an enlarged filament it may reach 0·3 mm. or more. Fusion of filaments laterally, however, was of somewhat rare occurrence and where

it had taken place was restricted to the fusion of the abfrontal ends of the filaments. A single filament in side view is shown in Figure 16, A (p. 510). Out-growth of the lobes of the ventral food grooves had occurred giving an irregular appearance to the ventral edges of the gills (Fig. 15), which appeared otherwise to be straight and uninjured.

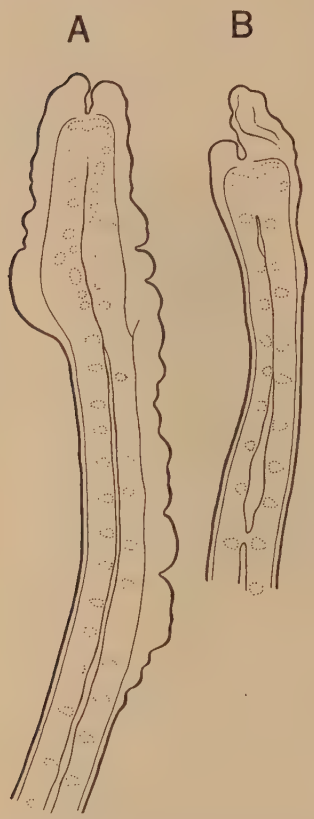

Fig. 16.—Lateral views of two filaments which have suffered enlargement of the frontal surface. B, shows extension of one side of the main ventral food groove. The fine inner line indicates the position of the lateral cilia. From preserved material. × ca. 18¼.

A mussel from the same locality on August 16th, 1928, had the gills in a similar condition, but the enlargement was restricted to a narrower zone (1·0 to 2·0 mm.) at the ventral edges.

The mussels, most unfortunately, were preserved whole in formalin, so that the fixation was poor, but transverse sections of one (Teignmouth, August 21st, 1928) showed certain points fairly clearly (Fig. 17, p. 511). The enlargement of the filament was confined mainly to the frontal face, for the position of the conspicuous lateral and latero-frontal rows of cilia was little changed; proliferation of the ciliated frontal epithelium appeared to have occurred, resulting in a much swollen frontal face with a corresponding enlargement of the internal canal (cf. the apical filaments of the two Eulamellibranchs *Pinna virgata* (**43**, Fig. 17, p. 214) and *Lima inflata* (**43**, Fig. 18, p. 216)). In the section sketched in Figure 17 there appears to be some interlamellar extension of the swollen filament, but this was not evident in all. Transverse sections of the enlarged gill filaments of this mussel showed a great irregular development of the frontal ends of the chitinous supports. While the chitinous supports of normal filaments stain quite darkly, this extra development of chitin is pale staining. Mucus cells were unusually well developed among the ciliated cells of the frontal epithelium; these were seen not only in sections (see Fig. 17) but in single filaments, stained with borax carmine and picro-nigrosin, and mounted whole.

Another mussel, also from Teignmouth (July 10th, 1928), showed slight irregular growth in the occasional extension of a filament beyond the free ventral edge of the gill (Fig. 16, B, p. 510) and in rare isolated groups of

FIG. 17.—Transverse section of filaments from the gill of a mussel from Teignmouth (the same individual from which Fig. 15 was taken), showing one much enlarged filament. As shown by the position of the lateral and latero-frontal cilia, the increased size is largely, or almost entirely, due to the great increase of the frontal surface. This filament has also extended in an interlamellar direction and has partly fused with an adjacent filament, which is only slightly enlarged: a mass of granular pigment is present at the junction of the two filaments. Especially striking is the development of the chitinous support of the enlarged filament; as indicated by the light shading this additional chitin is pale staining. A normal filament is shown on the right. Gland cells are present among the ciliated cells of the frontal epithelium. Formalin; Borax carmine and picro-nigrosin. × 210.

three or four filaments on the surface of the lamellæ as in Figure 18, A (p. 512). Very occasionally a mussel may be opened, in which, following a tear, the torn ends of the filaments have swollen or proliferated in an

FIG. 18.

A. Sketch showing a small area of enlargement of filaments—three filaments are chiefly concerned—on the surface of a gill of a mussel from Teignmouth, July 10, 1928.

B. Enlargement of the ends of filaments, presumably following a tear, with the formation of a new but irregular food groove. From the same gill as A.

A–B. From preserved material. × ca. 24½.

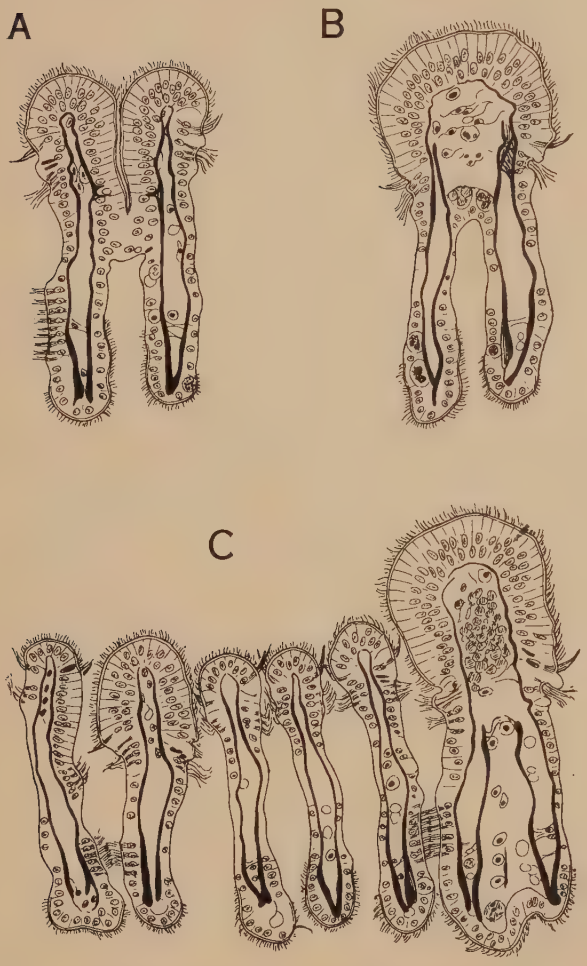

FIG. 19.—Transverse sections (from the same series as that of Fig. 20) showing filaments with abnormally deep cells towards the frontal face; one filament shows deep cells on one side only. To the right of C two fused filaments show a similar condition of the cells. A and B are sections of these same two filaments at a higher—more dorsal—level: in A the fusion is beginning, in B the frontal halves of the two filaments have fused, while in C the fusion is complete. In C a mass of granular pigment is present within the two fused filaments. From a mussel from North Bank, Fal Estuary, 1930. Bouin's fixative; Borax carmine and picro-nigrosin. × 210.

irregular manner while forming a new food groove, as had occurred on the gill of this mussel (Fig. 18, B).

Among the mussels received from the Fal Estuary in March, 1930, a single individual showed enlargement of the filaments. The ventral free edges of the gills were somewhat irregular in places, as though shallow pieces had been removed by injury, and the swollen extensions of the

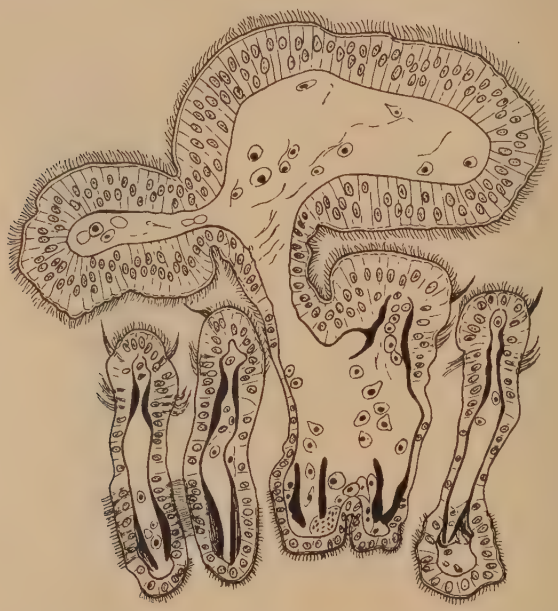

FIG. 20.—Transverse section of five filaments from the gill of a mussel from North Bank, Fal Estuary, 1930, showing a filament with greatly increased frontal surface and great depth of cells, fused with a filament on the right in which the frontal cells are also of great depth. There is no increase in the development of the chitinous supports (cf. Fig. 17). The separate filament on the right shows increased depth of the frontal cells of one side. (From the same series as that of Fig. 19.) Bouin's fixative; Borax carmine and picro-nigrosin. × 210.

ventral ends of occasional filaments increased the irregular appearance. The scattered groups of swollen filaments were mostly found near the ventral margins of the gills, but rarely the swelling was continued to almost half the width of the gill; the swellings had a much pitted appearance. Some of the lamellæ, however, also showed many of the filaments slightly widened, but otherwise normal. In transverse sections of such filaments it was seen that the widening was due to the unusually great depth of the cells towards the frontal part of the filament (Fig. 19, p. 513) (cf. the condition of the frontal epithelium near the ventral food

grove of normal filaments). Filaments were observed with the cells of one side of normal depth, while those of the opposite side were much deeper. Figure 19, A, B and C (right-hand part of section), which are sections through the same two filaments at different levels, show three stages in the gradual fusion of two filaments with enlarged frontal cells. Transverse sections of much enlarged gill filaments showed in this case, as in those from Teignmouth, that it was mostly proliferation of the frontal cells that had produced the swelling (Fig. 20, p. 514) : these cells were of abnormal depth, in some instances almost as deep again as those of the enlarged filaments of the Teignmouth mussels of August 21st, 1928 (cf. Figs. 17 and 20). There was no increased production of chitin, as in the Teignmouth example sectioned.

The enlargement of the gill filaments in the cases given was due no doubt to increased growth of the frontal epithelium dependent on some factor in the environment, e.g. decrease in salinity with consequent increased endosmosis. The abnormally great depth of the epithelial cells of the gill filaments of some mussels (as in Figs. 19 ; 20) is strongly suggestive of growth acceleration.

In the cases cited the enlargement was mostly of the frontal surface of the filaments, and little interlamellar extension was seen ; that it may occur, however, in such a situation in Mytilus was shown by the swollen out-growths in connexion with the interlamellar junctions in the gill of a mussel from the Fal Estuary.

The cilia on the swollen frontal surfaces beat normally and in general in a ventral direction, as shown by the movement of carmine particles.

(*d*) Concrescence of the Two Gills of One Side.

A Mytilus from Teignmouth (July 10th, 1928) showed a curious condition of the gills of the right side. Anteriorly, for about 7·0 mm., the two gills appeared to be fused side by side, and, since the outer one was somewhat shorter than the inner, the two ventral food grooves were at different levels ; immediately behind for about 7·0 mm. the two gills were fairly distinct : throughout this anterior region the gill filaments showed much crumpling. The gills then appeared to have fused again, so that in the middle region for about 18·0 mm. there was one "gill" only, but here it appeared to be formed of two lamellæ only —they could be clearly separated to within 3 mm. of the ventral edge, where they were connected by interlamellar junctions—and as both were provided with a dorsal food groove, they were presumably ascending lamellæ ; the gill was entirely free from the body. At this part the gill was about 7 mm. deep. While the gill filaments of the outer lamella appeared from surface view to be normal, those of the inner

lamella were in places bent somewhat out of the true dorso-ventral direction (i.e. crumpled), with some slight fusion side by side. For the remaining posterior 20·0 mm. the gills were normal and about 11·0 mm. deep. The shell had suffered no injury, and the inner surface of the valve was perfectly smooth.

A Mytilus from North Bank, Fal Estuary (March, 1930), had the gills of the left side, for about 5·0 mm. in the middle region, exceeding narrow (ca. 1·0 to 4·0 mm. deep), fused together, and entirely free from the body. So far as could be judged, the two ascending lamellæ (see **39**, Fig. 209, p. 229) alone were present in the fused portion as there was a dorsal food groove on both lamellæ. From the gill ridge hung short ends (ca. 1·0 mm. long) of the filaments of the two descending lamellæ. The left valve was occupied by a large blister, and though no hole had been bored through the shell in the region of shortness of the gill (Cliona borings were present in the umbo region), yet this condition was possibly due to injury, for the short ends of the descending filaments appeared to have been cut across. The case previously described may possibly have arisen from a condition such as this, as it is now known that regeneration of the gill may occur (see following note in this Journal, p. 551).

Peck (**37**, p. 50) gives an instance of abnormal concrescence in the gills of Anodonta. "In this case a torn portion of the inner gill-plate of the left side beyond the posterior edge of the root of the foot had become intimately adherent by concrescence to the inner surface of the inner gill-plate of the right side of the animal."

GENERAL CONDITION OF MYTILUS FROM THE VARIOUS LOCALITIES INVESTIGATED.

Fal Estuary.*

The mussels obtained from the Fal Estuary in 1927 (1648 examined from October 26th to November 25th) were mostly of small or medium size, ranging, however, from about 4·0 to 10·0 cm. in length, with but few of the large size, and were on the whole in rather poor condition, though the condition varied somewhat in different parts of the Estuary (see Table I). Not only were many of them poorly fished, but a certain number (6·6%) had large blisters and flat areas of wrinkled brown skin on the inner surface of the valves (Fig. 21, p. 519). Some of the blisters were so large that they covered the greater part of the valve and in certain cases projected into the cavity of the opposite one. The covering of such blisters varied greatly : in some it was a soft dark brown skin, in some a

* A chart of the Fal Estuary may be found in **36**, p. 3.

TABLE I.

CONDITION OF *Mytilus edulis* FROM VARIOUS LOCALITIES IN THE FAL ESTUARY.

Locality in Fal Estuary.	Date.	Number of mussels.	Number of mussels with Pinnotheres.	Number of mussels with Abnormal Gills.	Number of mussels with blisters in valves.	Remarks.
	1927					
Position not definitely known.	Oct. 26	250	17 (6·8%)	Numerous, but number not known.	9 (3·6%)	On the whole in rather poor condition. 4·0–9·8 cm. in length.
Position not definitely known.	Oct. 28	295	13 (4·4%)	59 (20%)	19 (6·44%)	On the whole in rather poor condition.
East Bank.	Nov. 2	279	9 (3·22%)	88 (31·54%)	23 (8·24%)	On the whole in poor condition.
Trelissick Reach.	Nov. 2	102	3 (2·94%)	19 (18·62%)	0	On the whole in good condition. Mostly about 5 or 6 cm. in length.
Turnaware Bar.	Nov. 10	9	0	2 (22·2%)	0	
Mylor Bank.	Nov. 23	195	13 (6·66%)	90 (46·15%) (*a*) 67 (*b*) 20 (*c*) 3	17 (8·72%)	Medium condition. 5·5–9·0 cm. long.
East Bank.	Nov. 24	254	10 (3·94%)	121 (47·64%) (*a*) 91 (*b*) 30 (*c*) 0	27 (10·63%)	Medium condition. 4·6–9·2 cm. long.
Parson's Bank.	Nov. 25	264	6 (2·27%)	97 (36·74%) (*a*) 74 (*b*) 23 (*c*) 0	14 (5·30%)	Well fished on the whole. Of rather larger size than those from East Bank.
	1930					
North Bank, between Mylor Pt. and St. Just Pt.	March 7	181	19 (10·5%)	72* (44·44%) (*a*) 22 (*b*) 44 (*c*) 6	26 (5 inhabited by Pinnotheres) (14·36%)	On the whole in fairly good condition. 5·4–12·4 cm. long.

(*a*) = Folding over of free ventral edge of the gill.
(*b*) = Fusion of filaments side by side.
(*c*) = Other abnormalities, including secondary grooves and folds, and simple narrowness of the gill.
* Out of total of 162 free from Pinnotheres.

layer of easily broken shell material, while in others it was so thick that it was difficult to pierce. The contents of those blisters examined, were in some instances almost clear liquid, in others mud, sometimes evil smelling. A certain number of the blisters were no doubt caused by the presence of *Polydora hoplura*,* which badly infested the shells. Cliona had attacked a number of the shells—generally the thicker part towards the umbo—and in a few instances apparently caused blisters.

Fig. 21.—Photographs of a selection of *Mytilus edulis* from the Estuaries of the Fal and Teign, showing blisters on the inner surface of the valves. The mantle has been entirely removed from the valves, except in E, where a triangular area of very thin mantle has been left on the blister. Various reductions.

A. Valve with round blister, which projects (about 8·0 mm.) into the opposite valve, in the posterior region, just ventral to the posterior adductor muscle. In opening the mussel the blister, which is of fairly thin shell, was cut: the dark line on the photograph, due to the cut, is on a level with the edge of the valve. Mussel 6·3 cm. long.

B. Valve with a blister of fairly thin shell material, entirely filling the anterior part of the valve in the mouth region. Mussel 7·5 cm. long.

C. Valve with blisters of fairly thin shell material almost entirely covering the surface. Antero-ventrally one of the blisters, though of shell material, has a wrinkled surface. The depth of the largest blister, measured at the spot where a small piece of the roof has been removed (small black area in photo), is about 11·5 mm. Mussel 9·2 cm. long.

D. Valve with a hard blister, convoluted in form, extending the length of the shell. Mussel 6·0 cm. long.

E. Valve with a large blister, occupying the greater portion, and projecting for about 8 mm. into the opposite valve. The shelly covering is extremely thin and in places has flaked off, exposing the brown skin beneath. The posterior adductor muscle has, to a certain extent, been encroached on by the blister. Towards the anterior and dorsal part of the shell there is a small flat area of dark brown skin. Mussel 7·1 cm. long.

F. Valve with a large smooth blister occupying much of the deeper portion, and reducing the depth of the valve in this region to about 4·0 to 6·0 mm. The blister, in this case, is covered by the shining nacreous layer and appears to be of considerable thickness, while in most other cases the shelly covering, which is somewhat thin, is of a greyish colour with a dull to rather dull surface. Mussel 7·7 cm. long.

G. Valve with almost the entire surface covered by an irregular low blister of dark brown skin. The shining spots on the surface of the blister would appear to be small areas of shell deposit. Posteriorly the blister has been removed from a round area exposing a worm aperture. Mussel 8·0 cm. long.

H. Valve with almost the entire surface covered with blisters, the covering of which is chiefly wrinkled brown skin; in places, however, the lower part of the wall is of very thin shell material. An L-shaped blister in the ventral and anterior part of the valve has had most of its roof removed. Mussel 7·1 cm. long.

I. A solid growth of shell material—judging by the weight—on the inner surface of the valve of a mussel from Teignmouth: it projects about 2·0 mm. beyond the level of the edge of the valve. The surface of the growth is of a dull dark grey colour and finely corrugated. Mussel 7·0 cm. long.

J. Occupying the middle and posterior part of the valve is a rather low irregular blister of hard shell material, with a ridged surface posteriorly. Towards the ventral edge of the valve is a blister of hard shell—except for a narrow irregular line along which it can be pierced by a needle—on a small base: it is about 9·0 mm. deep. Partly merged with the shadow cast by the latter is a small area of wrinkled dark brown skin. Mussel 8·0 cm. long.

* Identified by Mr. D. P. Wilson.

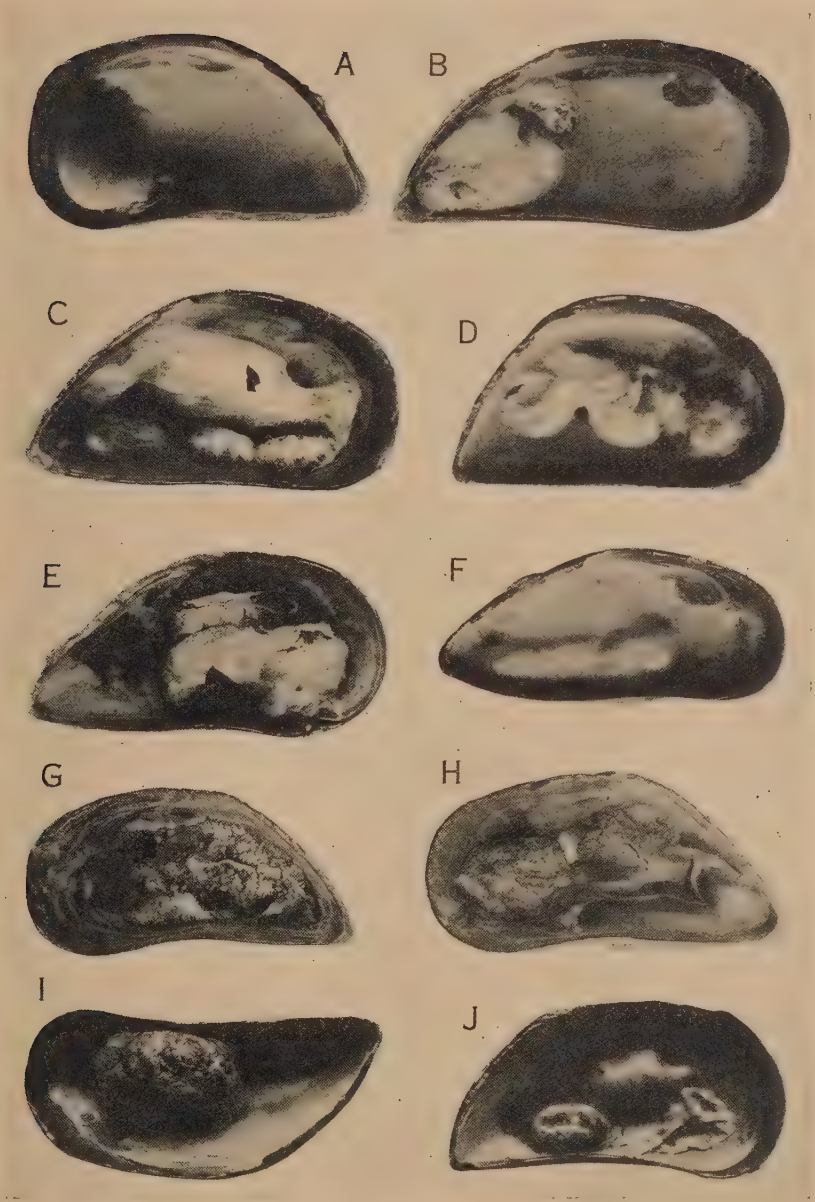

Fig. 21.

Many of the mussels attacked by Polydora showed—on the inner surface of the valves—raised but entirely closed in, tubular passages (see **28**, Fig. III, p. 41) covered with shell deposit of such a thickness that they could only be broken into with bone forceps ; in others, however, the covering was much thinner and in a few cases the tube of the worm projected through the thickness of the mantle into the mantle cavity, the apertures of the tube being entirely internal. A somewhat similar state of affairs has been described by Whitelegge in oysters of the coast of New South Wales attacked by *Polydora ciliata* (**47**, p. 48).

The presence of Polydora in a good percentage of the Fal Estuary mussels no doubt contributed to their poor condition. The abnormal condition of the gills, in individuals in which this took the form of folding, with reduction of the gill surface and disorganisation of the food grooves, no doubt also had an adverse effect on the general condition of the mussels.

In a certain number of the mussels there had been incomplete spawning, with irregular retention of the genital products in small masses projecting from the mantle surface. A peculiarity that was noticed in two specimens, was the presence of a horny, dark-coloured, ball in the byssus pit, while a third had a swollen mass, composed of many tiny, translucent balls, in the same position. It might be noted that among the batches of October 26th and 28th, a few mussels arrived with the mantle much swollen out with an accumulation of water between it and the face of the valve.

As it was desirable to know whether the abnormal conditions of the gills persisted, a further batch of mussels was obtained from the Fal Estuary on March 7th, 1930. These were examined with special reference to the state of the gills. Though the general impression gained was that the gills were not so badly abnormal as in those of 1927, yet the percentage with abnormal gills was quite high, namely, 44·4%. This may perhaps be explained by the fact that these mussels were examined especially for the state of the gills, while the previous batches obtained from the Estuary in 1927 were examined primarily for Pinnotheres : in a rapid inspection small areas of fusion of the filaments would be overlooked. In the March 1930 sample, mussels containing Pinnotheres of a size likely to cause injury have been omitted from the percentage with affected gills given above. While the percentage of mussels with abnormal gills is high, it is composed mostly of those with some degree of fusion of the filaments ; 22 only—out of a total of 72 with abnormal gills—had the ventral edge of the gill folded over, and in these the folding was not generally as extensive as in those of 1927.

The mussels of the 1930 batch varied in length between 5·4 and 12·4 cm., very few being over 9·0 cm. long. They were on the whole in fairly good condition, though a certain number, including those with highly abnormal

gills—with the surface much reduced and food grooves disorganised—were in poor condition.

Blisters were present in 26 mussels out of a total of 181 (14·36%). In some specimens they occurred in both valves, varying in number from one to three in each. The blisters were broken open and the contents examined : they mostly contained liquid with a varying quantity of mud ; in an exceptional case a piece of the mantle appeared to have been included. Worms were found in nine of the blisters, and in a further six the mud was in the form of fæces, indicating the previous presence of worms. Those found in seven of the blisters were one or more individuals of *Dodecaceria concharum*,* associated in one instance with three or four individuals of *Polydora ciliata*, while in two of the blisters Polydora was present alone. Some few shells were noticed with borings of *Cliona celata* in the thicker part of the shell near the umbo. Old borings of whelk-tingles were seen in some of the shells (about nine ; in two cases, one in each valve) ; they had been covered by shell deposit or skin, and in a few instances had given rise to large blisters. Very rarely blisters so formed were occupied by Dodecaceria or coated with *Cliona celata*,† these forms, no doubt, following the whelk-tingle. In three cases it was noticed that the outer gill of one side was extremely narrow or almost missing near the position of a former Gastropod boring ; the whelk-tingle, no doubt, had partly eaten the gill before being disturbed.

Estuaries of the Hamoaze, near Weir Point.

Mussels from this locality—1291 were examined between October 8th, 1927, and February 17th, 1928—were of varying size, from about 4·0 cm. to 12·0 cm. long ; the batches examined were fairly well fished, though the condition varied somewhat. A number had the shells covered with Halichondria. A certain amount of infection with Polydora occurred : in three cases it was noticed that the tube of the worm passed through the mantle so that the openings were entirely internal ; two of these contained *P. hoplura*, while nothing was found in the third. Mytilus with abnormal gills, other than those inhabited by Pinnotheres, were of rare occurrence.

Estuary of the Yealm.

The samples obtained from the Yealm Estuary—296 were examined between October 10th, 1927, and August 3rd, 1928—were composed of a good percentage of large specimens (about 7·0 to 12·0 cm. long), but the shape of the shell was very different from that of the Padstow mussels, which were also of large size, being of greater breadth in relation to the height. They were generally well fished, but the flesh inclined to be

* Identified by Mr. D. P. Wilson. † Identified by Mr. M. Burton.

yellowish. Very few mussels were noticed with abnormal gills, other than those infected with Pinnotheres, and the percentage of infected mussels was high.

Estuary of the Teign.

The mussels from the Teign Estuary —9262 were examined between December 6th, 1927, and February 26th, 1929—were mostly of small or medium size and on the whole rather poorly fished, though the condition varied somewhat with the different batches, roughly of about 1000 each ; those on January 4th, July 10th, July 19th, and August 21st, 1928, being in fairly good condition. Many were infested with Polydora, the inner surface of the valves showing raised, but covered in, tubular passages of the worms (see **28**, Fig. 111, p. 41). A small number had blisters in the valves ; one had a peculiar, large growth of shell in one valve, which, judging by the weight, was solid (Fig. 21, I, p. 519).

Two specimens, out of a total of 9262 mussels, were seen to be infested with the sporocysts and cercariæ (Bucephalus) of Gasterostomum.

About a dozen cases were noted of the presence of a horny ball in the byssus pit.

Mussels with abnormal gills were of exceptional occurrence, and in most of those noticed the abnormality consisted in enlargement of the gill filaments in certain areas.

It is of interest to note that Patella taken from mussels from this locality are a high shelled form (see also Report of the Council, Journ. Mar. Biol. Assoc., Vol. XVI (N.S.), p. 993, 1930).

The Estuary of the Camel, near St. Issey Cliff, Padstow.

The mussels from the Estuary at Padstow —10,866 were examined between November 8th, 1927, and August 9th, 1929—were fine specimens with smooth, clean shells, high and not very broad. The batches, of about 1000 each, contained a majority of medium and large specimens up to about 13·5 cm. in length. Throughout the period during which mussels were obtained, they were on the whole consistently well fished, with light-coloured flesh.

Blisters, in the valves of the Padstow mussels, were rare ; infection with Polydora was slight, and it was only exceptionally that the worm was found in small heaps of mud just inside the valves at the posterior end.

Infection by a species of Trematode (probably a new species*) occurred in about 235 out of the total of 10,866 mussels examined (2·16%), and seven cases were noted of infection by the sporocysts and cercariæ of Gasterostomum.

* The Trematode is being investigated at Leeds University.

Exceedingly few specimens —other than those infected by Pinnotheres or the Trematode—showed any abnormalities of the gills.

The Promenade Pier, Plymouth.

Two small batches only were examined from the Promenade Pier, one in December, 1927, of 232 mussels, and one in September, 1928, of 108 mussels, a total of 340 mussels. They were of small size : those of the former date were poorly fished, those of the latter date were well fished. A good number had the gills with jagged ventral edges, and three or so had the gills absent, or much reduced in length (dorso-ventrally), for a short distance, with a ridge in the mantle in this region. These have not been considered as abnormalities, as they were most probably due to injury by animals. A single Pinnotheres was found in each batch of mussels.

DISCUSSION.

The abnormalities of the gills of Mytilus, which have been described would appear to be correlated with some factor or combination of factors in the environment, and in the very great majority of cases not to be due to mechanical injury.

Mussels in estuarine, and especially in high estuarine situations, are subject to very fluctuating environmental conditions ; great salinity variations are known to occur, the quantity of detritus carried by the water would vary greatly, and possibly also the mineral constituents of the water and of the silt on the beds, temperature and other factors. Certainly the Fal Estuary mussels would appear to have been, and perhaps are still, subject to the influence of some adverse factor or factors which have upset the physiological processes, resulting in a high percentage of mussels with badly abnormal gills ; but without experimental work it is useless to attempt to attribute the abnormality of these mussels to any one factor. It is curious that mussels from other estuaries (see section, p. 516) were so little affected, and it would appear that conditions in the Fal Estuary differ markedly in some respect.

The various banks in the Fal Estuary* from which mussels were obtained are " mainly muddy, with, in places, top dressings of small shells which vary in amount in different parts " ; but the North Bank and southern part of the Mylor and East Banks, however, have an admixture of calcareous algal gravel, forming " a bottom of medium nature from a slightly muddy gravel, to a slightly gravelly mud " (see Orton, **36**, p. 73).

From the work of Orton (**35**, **36**) on the Fal Estuary oyster beds, it is known that the soil of the beds is rich in certain metals, and some of these

* A chart of the Fal Estuary may be found in **36**, p. 3.

may just possibly have an adverse effect on mussels. The soil on some of the beds, especially the Mylor Bank, contains an appreciable amount of copper (**36**, pp. 67, 70 ; **35**, p. 156), and it is noteworthy that Orton records that there was a distinct variation in the absorption of copper by oysters in March and May, 1921, and November, 1924. He suggests that this difference may mean " either that the copper in the bottom soil on Mylor Bank is becoming covered over, or is to some way losing its power to affect the oysters, or that oysters absorb copper differently at different seasons of the year " (**36**, pp. 67). Oysters apparently " can carry an unbelievable amount of copper in their tissues and still remain healthy in the sense that they are capable of reproduction " (**35**, p. 146). No mussels, however, from the Fal Estuary, or indeed from any other locality, have been noticed with any trace of greenness due to copper absorption, and it is possible that mussels are affected in a different way from oysters by the presence of copper, for Dodgson (**12**, p. 232) apparently found that copper salts had a deleterious effect on mussels, though this was probably in standing water.

Arsenic in somewhat large amounts—arsenic mines occur on the tributaries of the Fal—is also present in the silt on the banks (**35**, pp. 150, 153, 156, 159, 171). Its effect on mussels is unknown, but it may be noted that arsenic, as well as copper, mines were worked at one time also on the River Tamar, below Calstock—though they have been abandoned for some years—and mussels with abnormal gills are exceedingly rare from the beds near Weir Point, at the junction of the Tamar and Tavy.

Zinc occurs in appreciable amounts ; judging by the quantity present in oysters it would appear to be greatest in the locality of Restronguet Creek (**35**, p. 147). Zinc is apparently very toxic to mussels, at least, in standing water, for Dodgson (**12**, p. 140) found that it was impossible to use galvanised wire netting in cleansing experiments because " zinc was deleterious to the mussels, even to the extent of killing them." Under natural conditions, however, the toxicity of zinc to marine animals is probably very slight (see Orton, **35**, p. 147).

In an analysis of samples of soil from beds in the Fal Estuary, 100 to 1600 parts of arsenic, 16 to 240 parts of copper, 21 to 160 parts of zinc, and 20 to 40 parts of tin per million were found simultaneously (see Orton, **35**, p. 159).

The most comprehensive account of the physiology of the mussel is to be found in the section on " The Physiology of the Mussel with special reference to purification " in Dodgson's " Report on Mussel Purification " (**12**). He observed the conditions under which mussels will function normally, and one is impressed with their hardiness and tolerance to widely differing conditions. Their behaviour with regard to much silt in the water (**12**, p. 175), strength of tidal currents (p. 191), variations in

temperature (pp. 194, 198), and in salinity (pp. 208, 209), and lack of oxygen (p. 221) is dealt with in his work. The fact that mussels may exist for as long as 40 days under anærobic conditions, whilst ciliary action may persist for at least 25 days (**12**, p. 221), is especially interesting in view of conditions—such as temporary silting up of mud on the beds, sudden decrease in salinity, etc.—which might exist on the beds causing mussels to remain closed for long periods, with a possible adverse effect on the gills. According to Gray (**21**, p. 79), however, in closed mussels (removed from sea-water) ciliary movement would most probably be inhibited in two or three hours, owing to the concentration of CO_2 in the shell water, and thus the oxygen requirements of the animal would be reduced.

That mussels are tolerant of wide variations in salinity, and may even survive in fresh water, if the dilution of the sea-water be gradual, was shown as long ago as 1816 by Beudant (see Fredericq, **20**, p. 27), but it is well known that heavy mortality may be caused among mussels (**18**, p. 241)—and oysters (**36**, p. 69 ; **8**, p. 17)—in certain situations in estuaries by excessive freshness of the water due to exceptionally heavy rainfall. It is perhaps possible that sudden variations in salinity of an order not sufficient to cause death of the mussel, might yet adversely affect the gills. In spite of the great range of tolerance of the mussel, Flattely and Walton (**19**, p. 81) consider that there is undoubtedly a mean optimum salinity, and it is when exposed to this that the animal is capable of reaching its full development.

The Fal Estuary mussels were on the whole in poor condition, but it is impossible to say whether this was originally the cause or the effect of the abnormal conditions of the gills. As previously mentioned (p. 516), the mussels were badly infested with Polydora and to a less extent with Dodecaceria and Cliona, which in some cases had apparently caused the formation of large blisters in the valves, with general weakening of the mussels ; in some instances the mantle being nothing more than a thin, transparent skin. In this connection it is noteworthy that Daniel (**11**, p. 154) found, in comparing mussels deprived of food with mussels under normal conditions, that they had lost the power to control the water content of the tissue and that " In proportion to the total weight, the loss of water from the tissues of the mussels deprived of food is greater than that which occurs in the control mussels " (**11**, p. 158).

The factor or factors acting on the Fal Estuary mussels have apparently resulted in some cases in a tendency for the gill to collapse, the middle region—the part least supported—folding over longitudinally. Once folding had occurred, fusion of the folded over portion with the surface of the lamella beneath it would appear almost inevitably to follow. In other instances crumpling or puckering had arisen, most probably resulting in the crowding together of the filaments in certain areas, which, when

it became marked, would cause fusion of the filaments. It is curious that in gills which showed numerous areas of fusion it was frequently only one lamella of a gill which was affected, and that most usually the ascending lamella.

As is well known, concrescence is a common phenomenon among the Lamellibranchs, and various authors have given examples of variation—within the same species—in the concresence of gills with the visceral mass, with the mantle and between themselves (see Pelseneer, **38**, pp. 214, 215; Odhner, **32**, pp. 45, 48, 50, 53, 55, 60, 64; and Jackson, **25**, p. 326 footnote).

In certain Eulamellibranchs—such as *Cardium edule*, *Chama pellucida*, *Batissa tenebrosa*, *Psammobia vespertina*, *Donax serra*, etc.—where there is folding of the gill, Rice (**41**, p. 77) has described fusion of the gill filaments as a mechanical correlative of the folding of the lamellæ with consequent crowding of the filaments in certain regions; in this way explaining the presence of a greater number in the upper (dorsal) part of a fold than in the lower (ventral). Ridewood (**43**, p. 159) questioned whether the numerical discrepancy was not due to filamentar branching in the upper parts rather than to fusion in the lower parts of a fold. While perhaps fusion would seem more fully to satisfy the conditions, it is not apparent from Rice's sections through the gill of *Cardium edule* (**41**, Figs. 4–8, p. 79), whether it is fusion or branching. In the case of Mytilus the persistence of the separate chitinous supports, after fusion, would seem clearly to indicate that it is a case of fusion, and not of splitting or branching.

Rice (**41**, p. 78) found that in the Filibranchs—where the gill filaments are only loosely connected by ciliated discs—fusion of gill filaments side by side does not normally occur, even where the folding is extreme, the connexion being sufficiently loose to allow a considerable amount of play. In the light of Rice's findings, the widespread occurrence, among the Fal Estuary mussels, of varying degrees of fusion is of especial interest.

The enlargement of the gill filaments noted in five mussels from Teignmouth and one from North Bank, Fal Estuary, would appear to be due to some factor which has accelerated growth; the great depth of the cells in some parts is especially suggestive of this. In connexion with the abnormal growth of gill filaments in Mytilus, it is noteworthy that Ridewood (**43**, p. 174) in discussing the variation in extent of the interlamellar extensions of certain Lamellibranchs says, "Like so many features of gill structure this proneness of the filaments to extension in an interlamellar direction is of little, if any, systematic value. It is possibly related to the conditions under which the animal is living, and is the outcome of a permanently altered metabolism of the tissues of the gills. Perhaps it indicates abundant nutrition, or may be ascribable to increased temperature or diminished salinity of the water, or to the depth below the surface

at which the animal lives. Since, however, there is abundant subfilamentar tissue in *Unio pictorum* and *Psammobia pallida*, but little in *Unio ambiguus* and *Psammobia ferroensis*, one hesitates to frame generalisations."

One would imagine that Mytilus would not be prone to indulge in an abnormal growth of gill tissue, for Ridewood (**43**, p. 174) notes that "It is a significant fact that interlamellar junctions having the form of rods occur only in those genera with feeble development of subfilamentar tissue, viz. certain Filibranchia and Submytilacea."

That gill filaments may swell and retain the size after fixation was shown in one case, which is of interest in comparison with the enlarged gill filaments previously described (see p. 509). A Padstow mussel containing a Pinnotheres had shortness (dorso-ventrally) of the gill beneath the crab, and also six or so small groups of enlarged filaments on the face of the gill in this region. This gill was cut out and placed in a finger-bowl of the sea-water in general circulation in the tanks. After three days there was noticeable extension of the ends of some of the filaments along the ventral food groove, as well as swelling of certain of them in a narrow zone along the ventral margin. The cut ends of the filaments, where the gill had been detached from the body, also showed much enlargement. Compared with the swellings seen on the gill when the mussel was first opened, this secondary swelling appeared rather transparent, as though distended with water. The two conspicuous lines of ciliation—the lateral and latero-frontal lines of cilia—were more or less in their normal position in relation to one another, so that it appeared to be chiefly the sides of the filaments that had been blown out. Transverse sections through the swollen filaments are sketched in Fig. 22 (p. 528), and it will be seen that they have a very different appearance from those of Figures 17 (p. 511) and 20 (p. 514). This particular gill, beyond having the filaments somewhat roughly divided in attempting to separate them for examination, was otherwise treated in the same manner as numerous gills which did not undergo swelling. It might be noted that the chitinous supports were poorly developed, and that some few of the filaments were crowded with phagocytes. Later an unsuccessful attempt was made, by pulling filaments somewhat roughly apart, to induce swelling in other gills, when cut out of the mussel and placed in finger-bowls of sea-water. (The salinity of the sea-water in circulation (ca. $36^{\circ}/_{\circ\circ}$–$37^{\circ}/_{\circ\circ}$) is higher than that of normal sea-water (ca. $35^{\circ}/_{\circ\circ}$).)

Drew's (**14**) suggestion, that the intrafilamentar septum (in Pecten) is a brace to keep the filaments from swelling laterally owing to pressure of the blood, and in this way becoming circular and obstructing the flow of water between the filaments (see **10**, p. 44), is especially interesting in view of the swelling of the filaments of this gill. It may be noted that

difference of opinion exists as to the nature of the intrafilamentar septum ; various authors, including Ridewood (**43**, pp. 166, 168), holding it to be composed of chitin, while Kellog (**26**, p. 421) believes it to be endothelium (in *Pecten irradians*). Dakin (**10**, p. 43) found in *P. maximus* that it appeared to stain quite differently from the chitinous skeleton, appearing almost as if it were cellular, while nuclei, which were not adhering blood corpuscles, were seen in it. Setna (**45**, p. 376) states that in Pecten (three species), material fixed in Bouin and stained with Dobell's

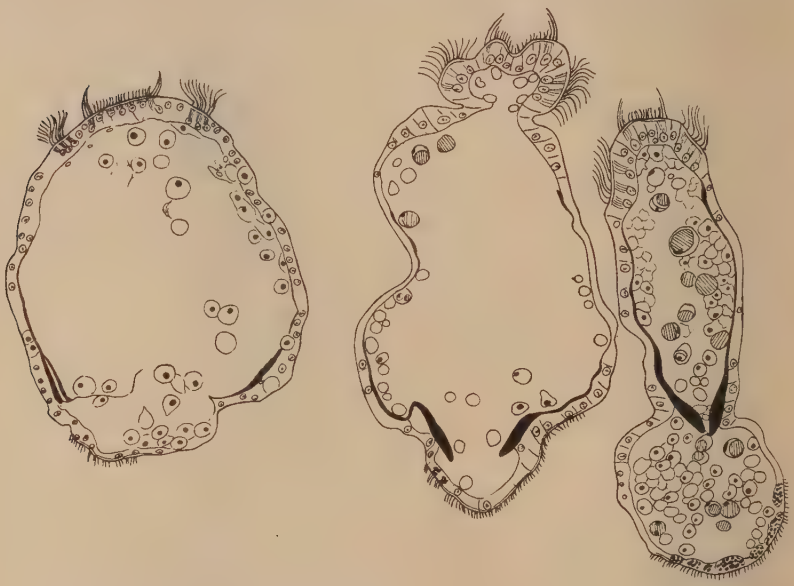

Fig. 22.—Transverse section of filaments from gill which showed increase in size of some of the filaments, after the gill had been cut from the mussel and left three days in a finger bowl of sea-water. The slight development of the chitinous supports and the numerous phagocytes in the filament on the right may be noted. The two filaments on the right are from a different part of the series. Bouin's fixative, Delafield's hæmatoxylin and eosin. × 210.

methyl-blue-eosin, while the chitin stains blue, the endothelial lining, as well as the intrafilamentar septum, stains red. This difference in colour staining between the chitinous supports and the intrafilamentar septum is also seen in *M. edulis* and *Modiolus modiolus* with Mann's methyl-blue-eosin and Mallory's Triple stain, after Bouin and Bouin-Dubosq fixation, but the difference is not so apparent with other stains such as borax carmine followed by picro-nigrosin (used by Ridewood). It is perhaps possible that the intrafilamentar septum may be composed of delicate muscle fibres.

That gill filaments may be increased in size to an alarming extent without rupturing was demonstrated by the active sporocysts of a species of Trematode within the lumen of the filaments of some few mussels from Padstow, causing the temporary enlargement of the filaments as they wormed their way along (Fig. 23). The Trematode possibly spreads

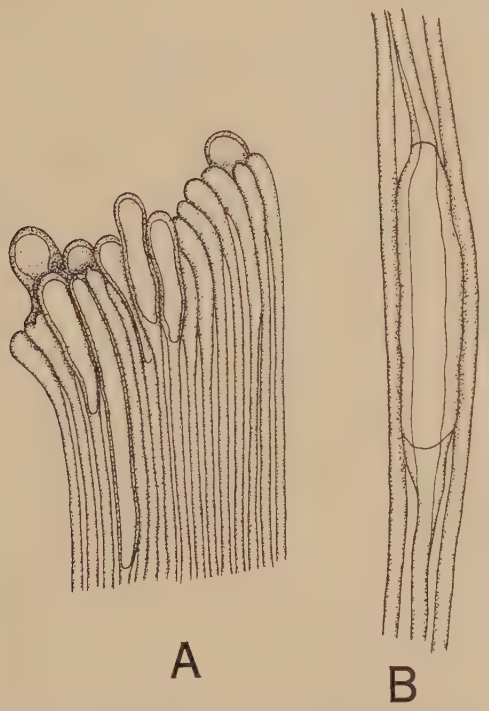

Fig. 23.—Sketches of living gill with sporocysts of a Trematode within the gill filaments. The main ventral food groove in A has an irregular appearance, due to the active sporocysts attempting to push forward. From both A and B it may be seen that the gill filaments recover their normal size after the passing of the Trematode. A, ca. 24⅓; B, ca. 76⅔.

through the mussel by way of the blood vessels ; in some instances they are seen crowded in the pallial vessels, causing them to assume a deep orange colour against the creamy white of the mantle ; the colour of the sporocysts varies, however, from cream to orange in different mussels. In badly infested mussels, the plicate canals (*organes godronnés*) of Sabatier, **44**) are packed with them, as are the mantle and parts of the visceral mass. The dorsal food grooves on the ascending lamellæ are

occasionally crowded with the active sporocysts and cercariæ of this Trematode, but on few occasions have they been seen actually within the gill filaments, though they have at times been noticed pushing their way along the interfilamentar spaces, in which case they may have actually burst through the gill epithelium, or may possibly have been individuals liberated in opening the mussel. When inside the filaments the active sporocysts would seem to tend to work their way towards the ventral free edge of the gill, where they cause extension of the lobes of the main food groove, giving the ventral edge of the gill an irregular appearance (Fig. 23, A, p. 529): and towards the dorsal food groove on the ascending

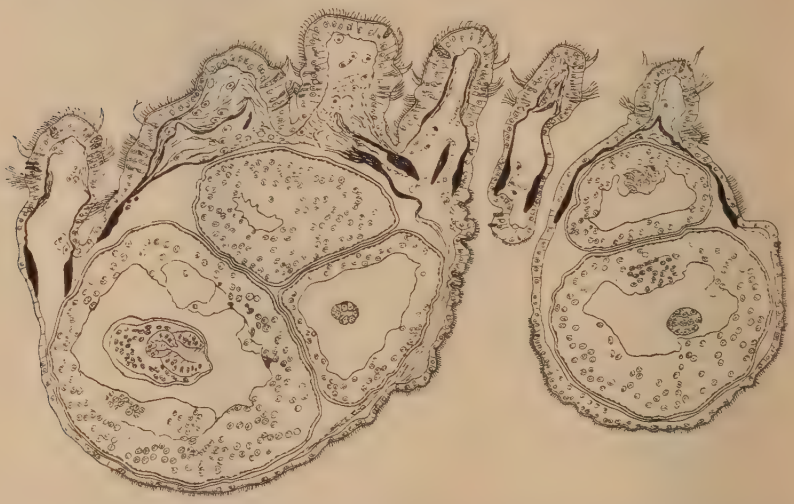

FIG. 24.—Transverse section of part of single lamella of a gill, near the dorsal food groove, showing great enlargement of the interlamellar (abfrontal) ends of the gill filaments owing to the presence of the sporocysts of a Trematode. In the filament on the right, two sporocysts are present side by side: on the left the enlargement of the gill filaments has apparently caused fusion. A cercaria is seen within one of the sporocysts. Bouin's fixative, Delafield hæmatoxylin and eosin. × 140.

lamella. The parasites can be seen to cause such great distension of the filaments that it is surprising they do not burst through the epithelium; it is manifest that the filaments are highly elastic as these resume the normal size after the passage of the parasite. The presence of the sporocysts gives to the filaments containing them an appearance somewhat similar to that described previously (p. 509, and Fig. 15); but the swelling is smooth and also the lines of latero-frontal cilia can be seen running across the swelling (Fig. 23. B), showing that the frontal epithelium experiences no more stretching—possibly less—than the rest of the epithelium. The extreme enlargement of the filaments is strikingly shown

in transverse sections, such as Figure 24, where there are two sporocysts side by side in the same filament. The enlargement of the area of ciliated abfrontal epithelium may be noted. This section also shows that fusion of the filaments may presumably be caused where swollen filaments are forced against each other. One would imagine that fusion from this cause is only likely to occur in areas where the active sporocysts tend to congregate for some time, e.g. near the ventral and dorsal edges of the gills. As mussels infected with the Trematode came from Padstow, where exceedingly little abnormality of the gills occurred among the mussels—apart from those infected with either Pinnotheres or the Trematode—the fusion in this case may be fairly safely attributed to the presence of the Trematode. Figure 25 shows

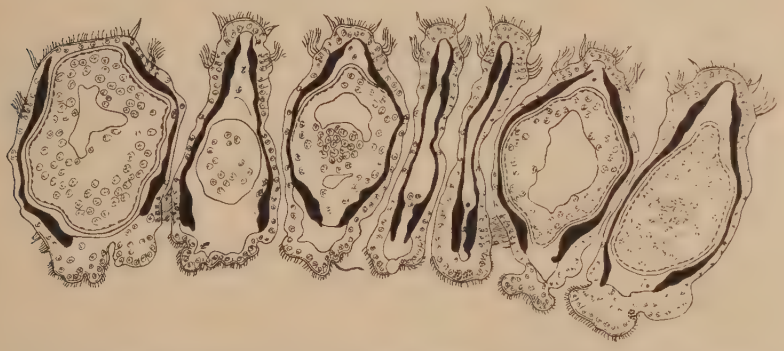

FIG. 25.—Transverse section of part of a single lamella of a gill, to show the crowding of filaments caused by the presence of sporocysts of a Trematode in five out of the seven gill filaments drawn. Bouin's fixative, Mann's methyl-blue-eosin. × ca. 171.

the crowding which resulted when five out of seven filaments, in the same lamella, each contained a small sporocyst.

The various abnormalities described would appear to support Rice's contention that "the lamellibranch gill is an extremely plastic organ, and one very liable to adaptive modification" (**42**, p. 76).

SUMMARY.

In the examination of a total of about twenty-four thousand mussels from various localities in Devon and Cornwall—estuaries of the Fal, Hamoaze, Yealm, Teign and Camel, and the Promenade Pier, Plymouth—it has been observed that those from various parts of the Fal Estuary included a high percentage of specimens with the gills in an abnormal condition, whereas such abnormalities were rare or absent in other

localities. The abnormalities are described from living and preserved material and consisted in the following types :

(*a*) Folding over of the free ventral edge of the gill, with concrescence.

Various stages in the degree of fusion have been observed, from slight fusion at the bend of the fold, to complete fusion of the folded over portion with the lamella beneath. Fusion and degeneration of the two inner arms of the fold, follows folding. The effect is noted of the reduction of gill surface, pumping power, and the disorganisation of the food grooves, consequent on folding, on the general condition of the mussel.

(*b*) Fusion of the gill filaments side by side.

This condition varied greatly in extent, from the fusion of two or more filaments side by side, to extensive areas of fusion scattered irregularly over the greater part of a lamella. Fusion was found to be confined almost entirely to one lamella, generally the ascending, the opposite one being little affected. It is suggested that fusion is due to crowding of the filaments, whatever may be the cause of the latter. The persistence of separate chitinous supports in the fused area, is considered as evidence that fusion has occurred, and not splitting or branching of the filaments.

(*c*) Enlargement of the gill filaments.

This rare form of abnormality was found in five mussels from the Teignmouth Estuary, and in one from the Fal Estuary. The enlargement of the filaments was mostly confined to a narrow zone at the free ventral edge of the gill, and appeared to be due to proliferation of the ciliated frontal epithelium, with a corresponding enlargement of the internal canal. In the mussel from the Fal Estuary the frontal cells of some of the gill filaments were of abnormally great depth : in the case of the gill sectioned from a Teignmouth mussel, great development of the frontal ends of the chitinous supports had occurred. It is suggested that the enlargement of the gill filaments may be due to some factor which has accelerated growth.

(*d*) Concrescence of the two gills of one side.

In two specimens of Mytilus it was noted that on one side, for a short distance in the middle region, one " gill " only was present, formed apparently of two ascending lamellæ and free from the body.

Brief notes are given of the general condition of Mytilus from the various localities investigated. The presence of blisters, in some instances of large size, in the valves of about 7% of the mussels from the Fal Estuary, and of a small number from the Teignmouth Estuary, is noted.

It is suggested that the abnormalities of the gills are correlated with some factor or combination of factors in the environment, information regarding which may be obtainable from further experimental observations.

METHOD OF DIVISION OF THE MANTLE CAVITY IN NORMAL INDIVIDUALS.

It is well known that in certain Lamellibranchs the division of the mantle cavity by the gills into supra- and infra-branchial chambers is not a morphological one, the union of the gills with the mantle, with the visceral mass, and with each other being a ciliary one. (See **39**, p. 228.) The actual method of connexion, however, has been described in but few forms. Herdman (**24**) described and figured it for *Margaritifera vulgaris*, living and well-preserved specimens of which showed apparent continuity of the walls of the supra-branchial chamber, slight pressure being needed for the separation of parts, when the ciliary nature of the junction was revealed. It might be noted that he remarks that in some, if not all, specimens, there was also some slight organic connexion between the two inner gills.

Ridewood described in *Anomia aculeata**—in which descending lamellæ alone are developed—the adhesion of the lower edges of the outer gills with the mantle, and the mutual adhesion of the lower edges of the two inner gills by means of patches of interlocking cilia (**43**, p. 194, and Fig. 8); while similar ciliary junctions have been described by Orton in Nucula (**33**, pp. 462, 463, 468, and Fig. 18) and in *Solenomya togata* (**34**, pp. 39, 41, 42, and Fig. 11), by means of which there is a complete division of the mantle cavity into supra- and infra-branchial chambers.

Dodgson (**12**, p. 171) gives diagrammatic representations of the boundaries of the supra- and infra-branchial chambers in *M. edulis* in three transverse planes.

In living Mytilus the normal ciliary junction between the gills and the mantle, and between the gills and the visceral mass, may be seen in individuals in which the posterior adductor muscle has been cut, if the two valves are separated only sufficiently for the line of junction to be seen. (The animal should be left to recover from shock.) It was found that the junction with the mantle was more likely to occur if the mantle—with the exception of the edge—were separated from the surface of the valve (this may be done by inserting the handle of a scalpel from the anterior end and carefully separating the mantle from the shell), so that it billows out somewhat, in this way compensating, in some degree, for the separation of the valves.

* Now *Heteranomia squamula*, see Winckworth, Proc. Malac. Soc., Vol. xv., pp. 32–4, 1922.

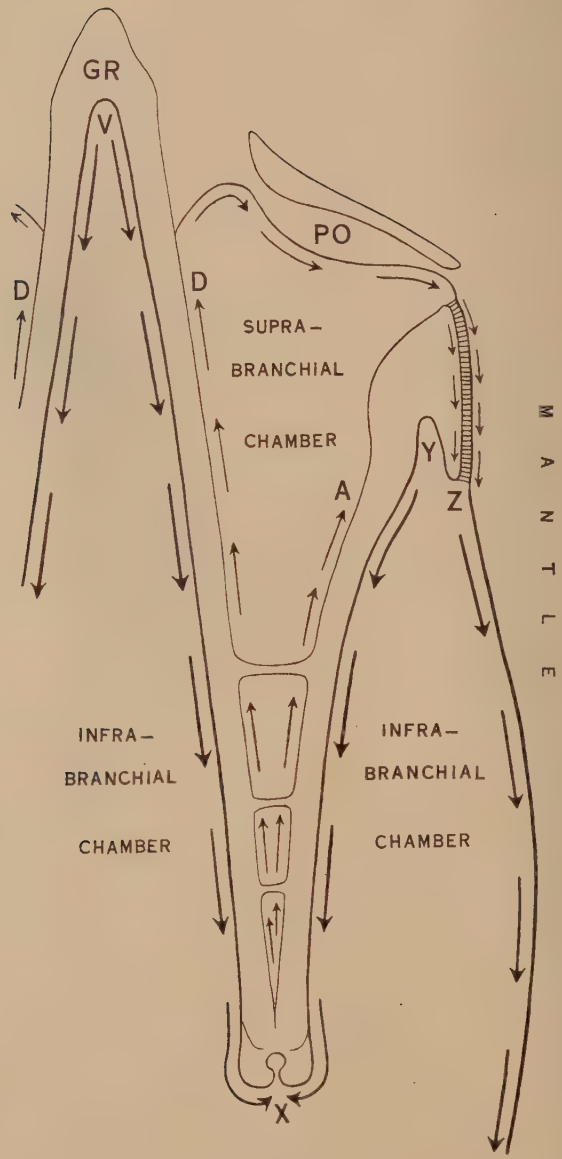
GR
V
PO
D
D
SUPRA–
BRANCHIAL
CHAMBER
A
Y
Z
MANTLE
INFRA–
BRANCHIAL
CHAMBER
INFRA–
BRANCHIAL
CHAMBER
X

Fig. 26.

In two individuals, in which the outer surface of the dorsal food groove of the ascending lamella of both outer gills made almost complete junction with the mantle, it was noted that gentle pressure on the gill caused slight raising of the mantle, which in one instance was very thin. In opened mussels the ciliary junction of gill and visceral mass was only seen for short stretches, owing to the visceral mass being damaged in opening.

The line of ciliary junction of the gill with the mantle in Mytilus is just ventral to the outer ends of the external plicate canals (Fig. 26), and investigation of the surface of the mantle in this region, by means of starch grains stained with iodine, showed that there is a narrow longitudinal zone, roughly about 1·0 mm. wide, over which the movement of particles is extremely slow, to the unaided eye and even with a lens the movement being almost indistinguishable ; particles, however, do move and tend to accumulate at the ventral edge of the zone, which by this means is shown to have a sharply marked ventral boundary. This boundary is usually visible in the living animal, owing to the lesser pigmentation of the zone in comparison with the rest of the mantle. Sections show that normally this region is not a ridge (see Fig. 6, A, p. 497) and may perhaps in specimens with well-developed gonad be slightly sunk. (It was found advisable to fix the mussel in the shell, in this way serious contraction of the mantle being prevented.) As food particles accumulate along the ventral boundary of the zone they get pushed into a narrow anteriorly directed ciliary current which runs just ventral to it (Fig. 26, Z), and from which they tend to be drawn into the ventrally directed ciliary currents over

FIG. 26.—*Mytilus edulis*. Diagrammatic representation of one outer division of the supra-branchial chamber, showing the ciliary junction between the outer surface of the dorsal food groove, Y, and the mantle ; and also the currents set up by the cilia on the walls of the chamber. The ciliary currents on the abfrontal surface of the filaments are shown as being directly dorsal, but actually they are somewhat posterior in direction (see Fig. 27). The ciliary currents on the frontal face of the filament and on the mantle are indicated by heavy arrows. The short arrows alongside the junction area, denote currents which occur over the interlocking cilia when the upturned edge of the gill is not adhering to the mantle.

GR, Gill ridge. PO, Plicate organ on roof of supra-branchial chamber.

A and D. Mark the position of posteriorly directed ciliary currents across the abfrontal surface of the ascending and descending lamella respectively, in the region where the ends of the filaments become fused together.

V. Marks the position of the anteriorly directed ciliary current between the bases of contiguous gills. The strength of this current appears to vary in different individuals, which may possibly explain why Kellog (**27**, p. 653) was unable to find it, though it has been observed by Orton (**33**, p. 460).

X. Marks the position of the anteriorly directed ciliary current along the main ventral food groove.

Y. Marks the position of the anteriorly directed ciliary current along the dorsal food groove on the ascending lamella.

Z. Marks the position of the narrow anteriorly directed ciliary current on the mantle. When the dorsal edge of the gill is touching the mantle, Y and Z form one anteriorly directed current.

the mantle region (see Orton, **33**, Fig. 11, p. 459, and Kellog, **27**, Fig. 18, p. 652); this, of course, only occurring when the gill is not adhering to the mantle. It is difficult to observe the appearance of the cilia clothing this tract in the living Mytilus, except in individuals in which the mantle is exceptionally thin, but in *Modiolus modiolus* where the mantle is normally very thin—not being invaded by the gonad in this species—a piece may be cut out and folded, so that the cilia are seen in side view. They closely resemble in appearance those of the ciliated discs on the lateral faces of the gill filaments, with which they agree in their interlocking function. In the living animal, as well as in sections, they have a stiff, regular appearance, as though they do not move far from the vertical during their beat, and can be easily distinguished from those on the mantle dorsal and ventral to them.

The position of the corresponding tracts of interlocking cilia on the visceral mass, may also be demonstrated by the use of starch grains stained with iodine, a longitudinal zone over which particles move extremely slowly, being visible beyond the inner ends of the internal plicate canals.

An examination of the outer face of the dorsal food groove on the ascending lamella of living Mytilus and Modiolus shows that this surface is clothed with cilia of the same appearance as those of the specialised tract on the mantle. A rather slow, somewhat irregular movement of particles occurs over this surface in a dorso-ventral direction, particles being drawn into the anteriorly directed current along the groove (Fig. 26, Y), from which there is a tendency for them eventually to pass on to the frontal face of the gill filaments. This, again, only occurs when the gill is not adhering to the mantle.

Sections show that the ciliated cells of the outer face of the dorsal food groove of the gill, and of the specialised tract on the mantle, have a marked border (see also Herdman, **24**, p. 227), formed by the basal granules.

The slow current over these surfaces may perhaps be sufficient to keep them clean, when the gill is not touching the mantle, and may transfer particles from the supra- to the infra-branchial chamber. When the gill is closely interlocked with the mantle or with the visceral mass, however, it is unlikely that any particles would be able to penetrate the barrier of interlaced cilia.

When the gill is interlocked with the mantle, the anteriorly directed current on the mantle forms, with that along the dorsal groove of the gill (Fig. 26, z and Y), a single anteriorly directed current. In this condition the current is more clearly defined, and there seems to be less tendency for particles to be drawn from the dorsal groove on to the frontal faces of the filaments, probably because the groove may be more widely open when gill edge and mantle are interlocked. The animal is able to alter the form

of the food groove considerably, and in specimens of Modiolus killed without narcotising, the outer wall of the groove may be reflected, so that the groove is non-existent ; while in Mytilus there is much contraction.

The abfrontal surface of the dorsal groove is abundantly supplied with scattered, very large cilia, and it is possible that they may have a tactile function, giving warning when the ascending lamella falls back on the descending lamella. These large cilia, which may occur singly, or two or three together, beat slowly, passing through 90°, and occasionally less than 90°, though this may be due to abnormal conditions during observation. Similar large cilia are also present among the short cilia on the abfrontal and frontal surfaces of the gill filaments ; and in addition occur among the ciliated cells of the plicate organs, on the surface of the mantle and the body, and on the palps (see also List **29**, p. 110) ; in fact, they probably occur on most external ciliated surfaces. On fixation they separate into their constituent fibres (see also List **29**, p. 110), and in sections, therefore, have the appearance of a tuft of cilia. These large cilia vary considerably in size, as may be seen on examining those on the abfrontal surface of a living gill-filament of Mytilus or Modiolus. In *Modiolus modiolus* they attain a great length, some times reaching 120μ. Gray, in a recent paper (**22**), has analysed the movement of these large cilia on the abfrontal surface of the gill filaments of Mytilus.

Contraction and expansion of the interlamellar junctions, which are first present some short distance ventral to the dorsal groove, along with the muscles of the dorsal groove itself, would most probably be sufficient to pull the gills away from the mantle, from the visceral mass, and from each other, causing temporary separation and approximation such as Dodgson (**12**, p. 170) described between the free dorsal edges of the ascending lamellæ of the inner gills in certain more or less moribund mussels. In this way the supra- and infra-branchial chambers may become continuous. As previously mentioned the dorsal grooves of the gills are provided with muscle-fibres, as seen in sections and shown by the curling up of pieces of dorsal groove cut from the gill.

In animals, which have been opened by cutting the posterior adductor muscle, movement of the gills is observable ; slight in the case of Mytilus (see also Pelseneer in **12**, p. 172), more evident in Modiolus. In the latter, the gills, from a position in which the ventral edges of the two gills of one side are touching, may separate until the angle between the descending lamellæ is 90° or more. Movements of separation and approximation of the gills, may be elicited, at least in Modiolus, by touching the frontal or abfrontal surface of the lamella with a needle. This movement may be compared with the concertina movement of the gills of Pecten, described by Setna (**45**, p. 370), but in Modiolus, while the whole gill is generally involved in the movement, the response is slower and not as certain, and

the movement slower than in Pecten. In Mytilus, the response to the touching of the gills is uncertain and the movement feeble. Such movements of separation and approximation of the gills, however, may also possibly contribute to the application of the dorsal free edges of the gills to the mantle, to the visceral mass, and to each other.

There is little difference in width between the outer surface of the dorsal groove of the gills (about 0·6 to 0·9 mm. wide, varying slightly in different regions and in different individuals) and the longitudinal tract of interlocking cilia on the mantle (about 1·0 mm. wide), so that practically no play of the dorsal free edge of the gill, in a dorso-ventral direction, is allowed for.

Although the junction of the gills with the mantle and the visceral mass in Mytilus and Modiolus would appear to be somewhat easily broken, it is probably of a less temporary nature than in the active Pecten. An opened mussel in which one of the outer gills had made complete junction with the mantle, was kept under observation for three hours, and throughout that time the junction remained unbroken, and was left in that condition when the watching ceased. In fact, observations point to the conclusion that, in mussels which have been opened, once the connection is made it tends to persist while the animal remains healthy, that is up to at least four days. It may be noted that Dodgson (**12**, p. 170) records that in a healthy mussel, under normal conditions, the free edges of the ascending inner lamellæ (in the posterior region) have always been seen to be in close apposition.

During the time the gill is connected with the mantle slight separation and approximation of the descending and ascending lamellæ occur, and, if the mantle has been freed from the shell, these movements may be sufficient to raise the mantle, exceedingly slightly.

In Pecten the temporary nature of the division of the mantle cavity has been observed by Orton (**33**, p. 461), who says "the upturned edges of the outer gill filaments touch the mantle during feeding, and in this way form at this point a temporary food groove." It may be noted that preparatory to the clapping of the valves the free posterior ends of the gills swing forward by contraction of the ctenidial muscles (see also **45**) and any connection between the gills and the mantle is broken.

Currents on the walls of the supra-branchial chamber.—In the supra-branchial chamber of Mytilus the main water current is posterior in direction (see Orton, **33**, Fig. 11, p. 459), and most of the surface currents on the walls of the chamber have also a posterior tendency, but have not previously been described.

The plicate canals are found on the roof of the supra-branchial chamber, the width of each division of the chamber being roughly the length of these organs. The external surfaces of the canals are highly ciliated,

rapid currents passing over them, in the main, in a dorso-ventral direction (see Fig. 26). These canals, to which Sabatier gave the name of *organes godronnés* (**44**, p. 56), have a respiratory function.

In Mytilus the cilia on the abfrontal faces of the filaments beat chiefly in a dorsal direction, but actually obliquely across the filaments, so that particles pass across the abfrontal surfaces of the lamellæ in a direction

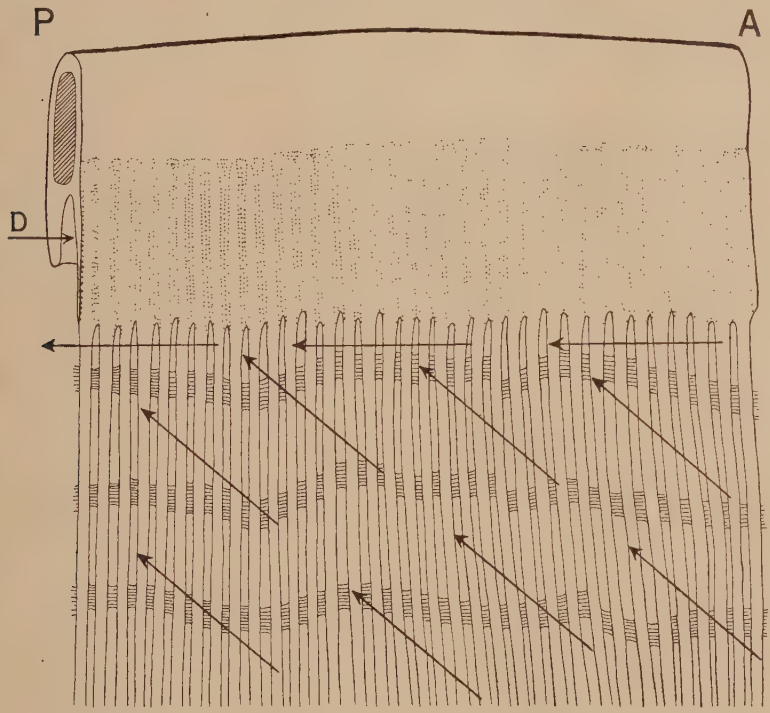

FIG. 27.—Mytilus. View of the abfrontal surface of an ascending lamella in the region of the dorsal food groove, to show the ciliary currents.

A and P. Anterior and posterior.

D. Arrow showing the direction of the current along the dorsal food groove. The narrow stippled areas indicate the chitinous supports in the region where the ends of the filaments are fused together. × ca. 41½.

which is anterior-ventral to posterior-dorsal (Fig. 27). Across the dorsal ends of the gill filaments—in the region where they become fused together —of both descending and ascending lamellæ, the cilia maintain a posteriorly directed current (Figs. 26, D and A; 27). In most of the individuals which were examined the epithelium covering the fused ends of the filaments was either feebly ciliated, or destitute of cilia with the exception of scattered very large cilia (see p. 537), and there

was only occasional slight movement dorsalwards of particles jerked forward by the latter: in such individuals the posterior ciliary current across the base of the filaments was very clear. Very rarely individuals were seen in which the abfrontal cilia were continued on to the epithelium covering the fused ends of the filaments; there was in consequence a ciliary current across this surface, in a postero-dorsal direction, and the purely posteriorly directed ciliary current was not then as clear.

In the few specimens of *Modiolus modiolus* examined, the abfrontal cilia of the gill filaments were very poorly developed or absent, except for large scattered cilia, and the ciliary currents on the abfrontal surfaces of the lamellæ were therefore weak or absent.

The greater part of the material for the work recorded in this paper was obtained during the tenure of a research studentship of Bedford College, University of London. I wish again to express my gratitude to the Director and Council of the Marine Biological Association for allowing me to work at their Laboratory at Plymouth, and to the London University for granting me the use of their table. For the composite photograph of Figure 21 I am indebted to Mr. A. J. Smith. My sincere thanks are due to Prof. J. H. Orton for reading the manuscript, and for helpful criticism and advice.

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Note on Some Abnormalities of Labial Palps and Foot of *Mytilus edulis.*

By

D. Atkins, B.Sc.

With 7 Figures in the Text.

In view of Pelseneer's work on " Les Variations et leur Hérédité chez les Mollusques,"* it seems worth while to record briefly certain observed abnormalities of the labial palps and the foot of *Mytilus edulis*. Among some thousands of specimens examined, however, such conditions were rare, and it is probable that at least some of them arose through injury.

Labial Palps.

The abnormality of the palps most generally met with is evidently the result of natural regeneration, following injury to the tips of the palps. From the injured surface tiny outgrowths arise, usually three in number, two of which appear to have a common base. Figures 1, A–C, shew different degrees of regeneration; two examples have been seen in approximately similar conditions. (The normal tip of the palp may be seen at P in Figure 2.)

Pelseneer (p. 181) records the finding by Sykes of a specimen of *Tellina incarnata* in which the palps were missing, but gives no further examples of variation in palps. However, his Figure 121 (p. 204) of a gill of *Boreochiton marginatus* with trifurcated tip shows a similar state of affairs to that recorded in this note for the palps of *M. edulis*.

Bifurcation of the tip of the palp has been observed in one or two instances.

The right inner palp of a Mytilus from Padstow showed two small accessory palps near the base (Figure 2); these had a common base, only the tips, which pointed in opposite directions, being free. In preservation the tips have become curled, and that of the more proximal secondary palp is hidden beneath that of the more distal one. A second example of this type of accessory palps was noted on the right inner palp of another Mytilus from the same locality, but in this instance they originated about

* P. Pelseneer, Les Variations et leur Hérédité chez les Mollusques. Acad. Roy. Bel. Mém., 2nd sér., Tome V, 1920.

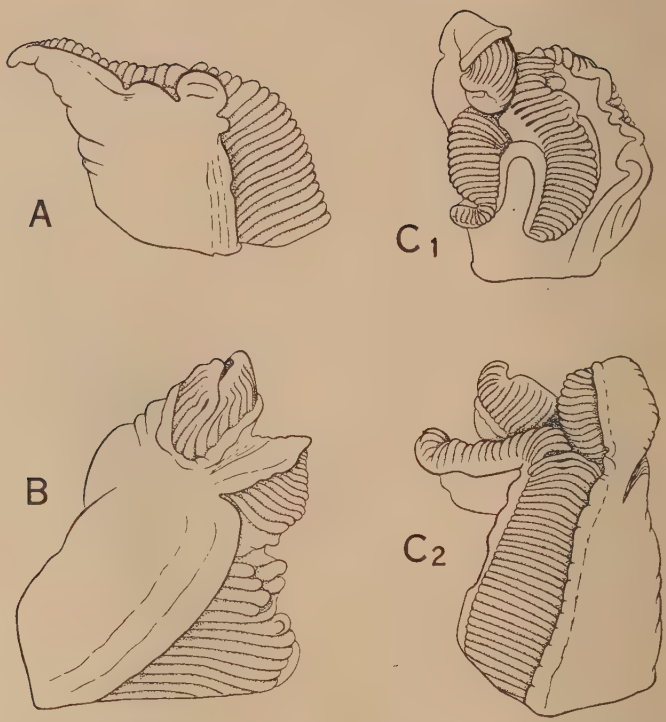

FIG. 1, A–C.—*Mytilus edulis*. Sketches showing stages in the formation of outgrowths from the tips of three palps, A, being the youngest and C, the oldest stage. In C1, the trifurcated tip of the palp has become folded back on to the smooth outer surface during fixation; in C2, it has been forcibly flattened out. × ca. 6¼.

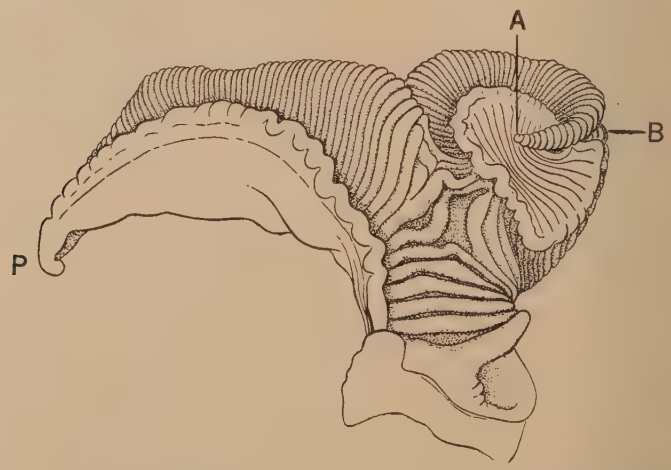

FIG. 2.—*M. edulis*. Sketch of palp with two accessory palps near the base. These have a common base, the tips only being free: that of the more proximal one, B, is hidden beneath the other, A. P, tip of principal palp. × ca. 6¼.

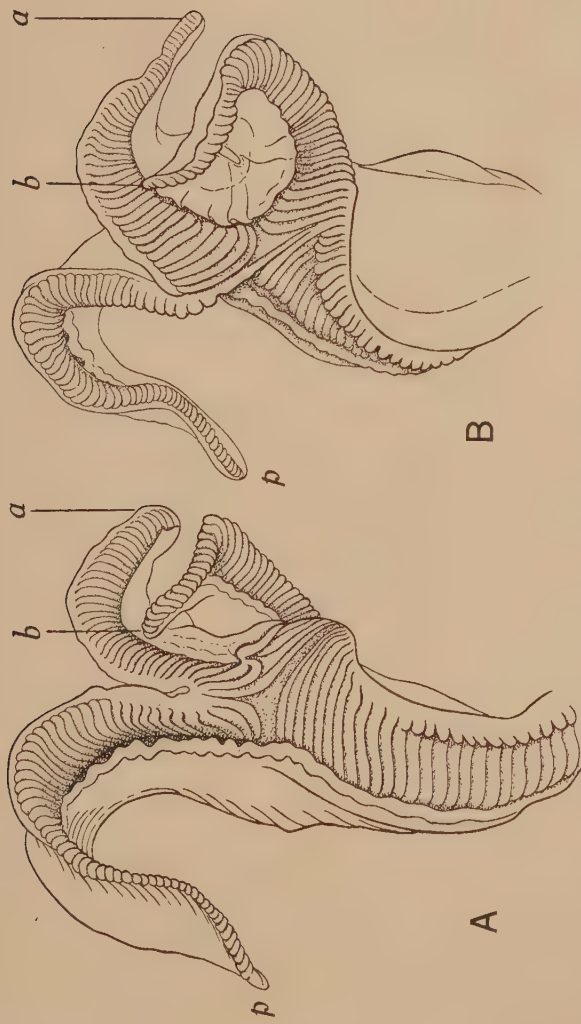

FIG. 3.—*M. edulis*. Different views (A and B) of two accessory palps, *a* and *b*, originating from about midway along the length of the principal palp. *p*, tip of principal palp. × ca. 6¼.

midway along the length of the principal palp, and were more highly developed (Figure 3).

A tiny accessory palp originating from near the base of a left inner palp,

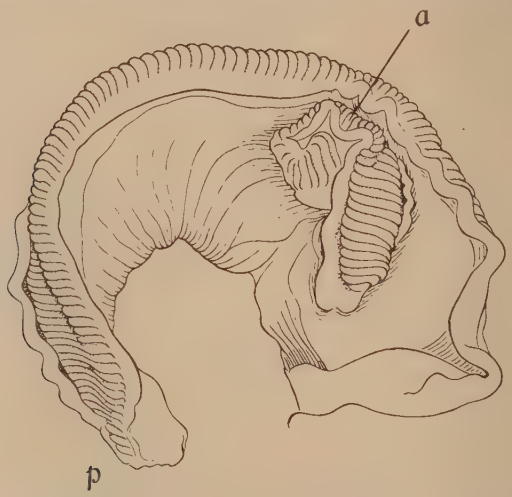

FIG. 4.—*M. edulis*. Palp with a small accessory palp, *a*, near the base on the smooth outer surface. *p*, tip of principal palp. × ca. 6¼.

on its smooth outer surface, is shown in Figure 4. In this instance the difference in size of the principal and secondary palp is marked.

FOOT.

Several different types of foot abnormality have been noted in *M. edulis*. That most generally met with was a small foot-like outgrowth, or rudimentary accessory foot, originating from near the base of the primary foot, the latter showing no injury to its tip. The outgrowth was lateral, but somewhat dorsal in position (see Figure 5), and in three cases was on the right side and in one on the left. The outgrowths varied somewhat in size; that sketched in Figure 5 being the largest seen. These foot-like outgrowths were pigmented a dark brown like the primary foot, but were without a ventral groove or anterior sucker. A single case was noted of a similar tiny outgrowth about midway along the length of the foot and distinctly on the lateral (right) edge. Pelseneer figures (Figure 94 *bis*, p. 133) a somewhat similar outgrowth on the right side of the foot of *Cyclas cornea*, which, however, was spheroidal.

An interesting case of foot abnormality was that sketched in Figure 6, where the accessory foot was ventral in position though slightly lateral, and the ventral groove of the main foot divided, the secondary foot in this instance being provided with a ventral groove.

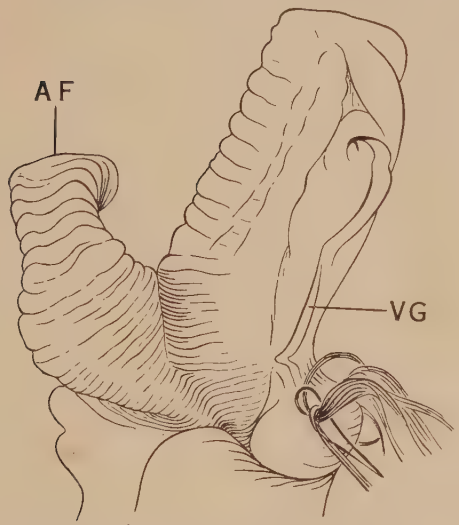

FIG. 5.—*M. edulis*. Foot with a small foot, AF, originating from near the base. VG, ventral groove of primary foot. × ca. 6¼.

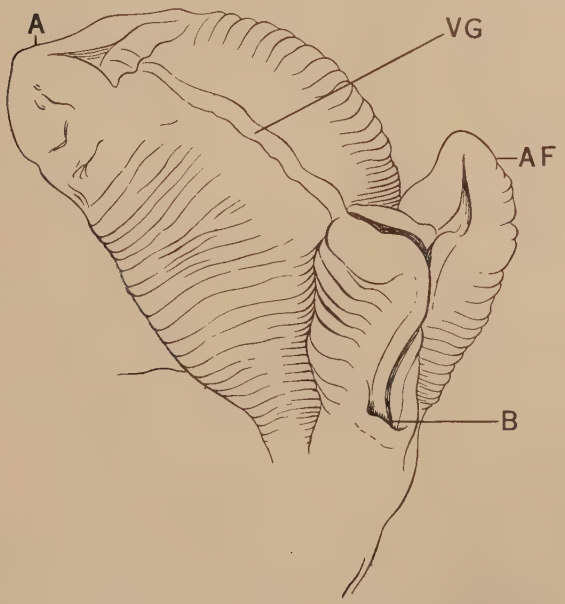

FIG. 6.—*M. edulis*. Foot with an accessory foot, AF, which is ventral in position and is provided with a ventral groove. A, tip, and VG, ventral groove of primary foot; B, byssus pit. × ca. 6¼.

One Mytilus was noted in which the tip of the foot was missing, the foot being obviously injured and discoloured (Figure 7). There was a slight irregular outgrowth from the main foot anteriorly, but what is of especial interest was the presence of tiny foot-like protuberances, one on either side of, and seated on the anterior retractor muscles, that on the left being slightly more anterior in position than that on the right. These

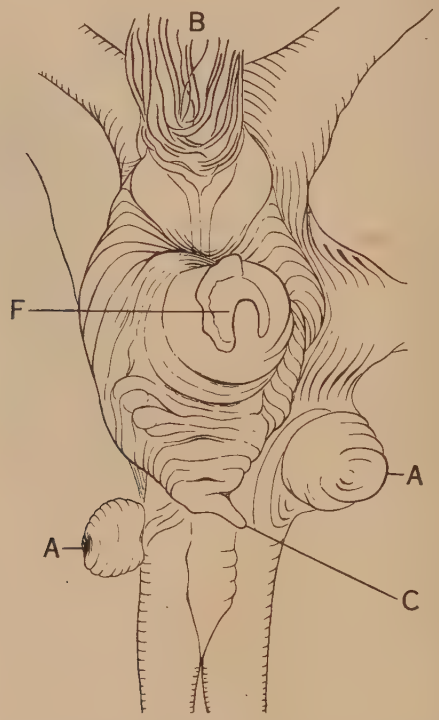

Fig. 7.—Sketch of foot of *M. edulis*, showing the tip missing as a result of injury, and two small foot-like outgrowths, A, A, one on either side of the anterior retractor muscles. F, injured tip of primary foot; C, small outgrowth from primary foot; B, byssus. × ca. $6\frac{1}{4}$.

were pigmented, but appeared to have no ventral groove. The figure shows them in a much contracted condition. As the foot is protruded from the shell in travelling, and during the spinning of the byssus, it is liable to suffer injury (see also Pelseneer, p. 133), as has evidently occurred in this specimen, but it is doubtful whether the foot-like outgrowths from near the base of the foot are due to this cause.

The position of the foot has been noticed to vary somewhat, in one instance being very anteriorly placed.

Note on the Regeneration of the Gill of *Mytilus edulis.*

By

D. Atkins, B.Sc.

With 8 Figures in the Text.

Introduction.

In connexion with work on reversal of the frontal cilia on the gill filaments of *Mytilus edulis* (**1**), it became desirable to know whether the gills were capable of regeneration after injury. With the exception of the peculiar method of growth or regeneration of the incubatory gills in Cyclas, briefly described by Poyarkoff (**11**), which, even if his preliminary observations are confirmed, would hardly appear to be true regeneration,* the only reference that can be found on the subject of regeneration of the gills in Lamellibranchs is that in the paper by Bloomer (**3**) on malformed specimens of *Anodonta cygnea*, where the condition was apparently due to injury : he concluded " that the animal is able to repair even extensive damage to the mantle-lobes, but is not able to make good injuries to the gills " (**3**, p. 138).

As *M. edulis* is not infrequently found having the gills with very jagged ventral edges, and a few specimens even with the gills more or less entirely missing for a short length—in some instances almost certainly attributable to injury by boring whelk-tingles—it was thought that regeneration of gill filaments possibly might not occur in Mytilus. A single specimen experimented on in June, 1929, showed no signs, after 112 days, of regenerating the wedge-shaped pieces cut from the gills, beyond the formation of a food groove at the cut ends (see **1**, p. 960). From further experiments, however, it is evident that regeneration of the gills of *M. edulis* may occur.

EXPERIMENTS.

Mussels with strong shells (ca. 7·0–8·0 cm. long) were chosen for the experiments, so that the shells would not be likely to fracture easily on being forced open. The ease or difficulty with which the valves could be

* " La formation des sacs d'incubation doit gêner considérablement le développement normal du feuillet réfléchi de la lame branchiale interne. En revanche ce feuillet s'accroît d'une façon si singulière que je qualifierai ce cas de régénération de ce feuillet bien que sans doute l'animal n'en perde en réalité aucune portion " (**11**, p. cxxxvi).

forced apart was taken as an indication of the condition of the mussel; those of which the valves could be easily forced apart were rejected.

The shell was first opened slightly with an oyster opener; the pointed and thin side of a wooden wedge inserted; worked further in and slowly twisted, until the mussel was forced open to the greatest width of the wedge (about 1·0 to 1·5 cm.). It was done gradually so as to strain the muscles as little as possible. In spite of care the shell fractured badly in some few instances, and the mussels had to be discarded.

The injuries were made near the posterior end of the gills,* owing to the difficulty of reaching any other part. So far as could be seen, the gills of all the mussels used were normal.

The experimental mussels were placed separately in finger bowls, and the bowls sunk in one of the glass-fronted tanks in the general circulation in the Plymouth Laboratory, the tank used being in the shade.† An average of two to four litres of tow nettings a day were tipped into the tank.

Temperature readings of the water in the experimental tank were not taken, but the highest and lowest morning readings (taken at about 9.30 a.m.) for each month, of water temperatures of a similar tank, three tanks away and on the same side of the building, are as follows:

	Max. °C.	Min. °C.
Dec., 1929	12·1	10·8
Jan., 1930	10·8	9·4
Feb.	9·7	7·1
March	10·3	8·4
April	11·9	9·7
May	13·2	10·7
June	15·4	13·2
July	16·4	14·9
Aug.	16·8	15·0
Sept.	17·2	14·1
Oct. 1–17	15·1	12·6

(These figures have been abstracted from the temperature readings taken daily by Mr. A. J. Smith.)

The experiments were started on November 29th and December 1st, 1929, and in February, two out of the ten experimental mussels were found to have died. Five individuals (A, C, D, F, K in Table I) were opened on July 30th, 1930; of the remaining three, one (E) was found gaping on September 15th, the two survivors (B and J) were opened on October 17th, 1930.

Notes on the experimental mussels are given in Table I.

* For convenience in description the two demibranchs on each side of the body are considered as two gills.

† Coulthard (**6**, p. 136) finds that "Mussels display maximum growth in approximately 50% sunlight, slightly less in darkness, and least in full sunlight."

TABLE I.

EXPERIMENTS ON REGENERATION IN THE GILL OF *MYTILUS EDULIS*.

(All experiments were begun on either Nov. 29th or Dec. 1st, 1929.)

Mussel.	Date of opening in 1930.	Condition on date of opening.	Nature of injury inflicted.	Result.
A.	July 30	A good deal of byssus formed ; strongly attached. Mussel rather thin. Gills markedly pigmented.	(*a*) Right inner gill.—Wedge-shaped piece cut out of ventral edge in such a way as to leave some filaments unattached except by ciliated discs.	(*a*) Regeneration both of food groove and filaments occurred (see Fig. 1).
			(*b*) Left inner gill.—Two slanting cuts with scissors from ventral edge of gill in a dorsal and anterior direction, so as to isolate two pieces of gill except for attachment by ciliated discs.	(*b*) Pieces sloughed off : regeneration of food groove and of filaments followed (see Fig. 3).
			(*c*) Left outer gill.—Operation as for left inner gill.	(*c*) Pieces sloughed off : regeneration of food groove and of filaments followed (see Fig. 2).
B.	Oct. 17	Rather poorly fished, but valves fairly stiff to force apart. Gills only slightly pigmented.	(*a*) Right outer gill.—Injured by pulling some of the filaments with forceps.	(*a*) Regeneration of food groove occurred. Injury showed as two long inlets in gill, with some slight abnormality of filaments.
			(*b*) Right inner gill.—Shallow piece cut out of ventral edge.	(*b*) Regeneration of food groove occurred, also some very slight regeneration, with apparent fusion, of filaments.
			(*c*) Left inner gill.—A slanting cut with scissors from ventral edge in a dorsal and anterior direction, so as to isolate a piece of gill except for attachment by ciliated disc.	(*c*) Most of piece sloughed off. Little, if any, regeneration beyond formation of new food groove. At deep end of cut some irregular joining up of cut ends of filaments. Five or so short lengths of filaments separated dorsally from gill by a tiny space, but attached anteriorly and posteriorly.
			(*d*) Left outer gill.—Piece cut out of ventral edge.	(*d*) Regeneration of food groove occurred, also some slight regeneration, with apparent fusion, of filaments (see Fig. 5).

TABLE I—(*continued*).

Mussel.	Date of opening in 1930.	Condition on date of opening.	Nature of injury inflicted.	Result.
C.	July 30	Much byssus formed; strongly attached. Fairly well fished. Gills very slightly pigmented, with practically no intensity of pigment along ventral edges.	All four gills cut obliquely with scalpel, the cuts not reaching the ventral edge. In some cases the gill was supported by the blade of an oyster opener (see p. 557).	No injury apparent when gills examined with microscope. Regeneration of epithelium occurred, and in cases where filaments had been cut through, fusion of cut ends had also taken place.
D.	July 30	Much byssus formed; strongly attached. Mussel in good condition.	As for C.	As for C.
E.	Sept. 15	Valves gaping. Mussel thin.	As for C.	No injury apparent when gills examined with microscope; but a small piece missing from the ventral edge of left inner gill; one of the cuts may have been too near the edge of gill. Regeneration of epithelium had occurred, and in cases where filaments had been cut through, fusion of cut ends had also taken place.
F.	July 30	Much byssus formed; strongly attached. Mussel in good condition. Spawned as female, after being opened and put in finger bowl under circulation.	All four gills cut obliquely with scalpel, the cuts not reaching the ventral edge. In all instances the gill was supported by the blade of an oyster opener (see p. 557).	No injury apparent when gills examined with microscope. Regeneration of epithelium had occurred, and in cases where filaments had been cut through, fusion of cut ends had also taken place.
G.	Feb. 20	Found dead.		
H.		Found dead.		

J.	Oct. 17	Poorly fished, valves easily forced apart. Gills very slightly pigmented.	(*a*) Right inner gill.—A slanting cut with scissors from ventral edge in a dorsal and anterior direction, so as to isolate a piece of gill except for attachment by ciliated discs.	(*a*) Piece sloughed off. Cut made at extreme posterior end of gill, very close to dorsal food groove; as all interlamellar junctions removed, the lamellæ separated, and the cut ventral ends of each have rounded off independently, with irregular edges and *without* formation of food groove.
			(*b*) Right outer gill.—Cut obliquely with scalpel in several places, cuts not reaching the ventral edge. Gill not supported (see p. 557).	(*b*) No sign of injury when gills examined with microscope. Regeneration of epithelium had occurred.
			(*c*) Left inner gill.—As for right outer gill (*b*), but gill supported by blade of oyster opener (see p. 557).	(*c*) No sign of injury when gills examined with microscope. Regeneration of epithelium had occurred, and in cases where filaments had been cut through, fusion of cut ends had also taken place.
			(*d*) Left outer gill.—Large piece cut out of ventral edge.	(*d*) Regeneration of food groove occurred, also some slight regeneration, with apparent fusion, of filaments.
K.	July 30	Valves gaping somewhat; would not close when touched. Mussel in poor condition. Gills rather slightly pigmented, but with a darkly pigmented (brown) ventral edge.	(*a*) Right outer gill.—Large piece cut out of ventral edge.	(*a*) Regeneration of food groove occurred, but no appreciable regeneration of filaments. New food groove was very noticeable as it was almost unpigmented, while that on old part of gill was very darkly pigmented. The gap was somewhat smaller than the piece removed, owing to the inward bending of the filaments at either end.
			(*b*) Right inner gill.—Piece cut out of ventral edge.	(*b*) As for right outer gill.
			(*c*) Left inner gill.—Piece cut out of ventral edge.	(*c*) As for right outer gill.

REGENERATION.

Regeneration of the gill of Mytilus consists of the formation of (*a*) food groove and (*b*) gill filaments. These may be considered separately, as the former may occur without any appreciable regeneration in length of the gill filaments.

(*a*) Food Grove.

A food groove was formed at the cut edge of the gill in all cases, with but one exception, and is apparently always formed, if the cut edges of the filaments of the descending lamella are able to touch and so to fuse with those of the ascending lamella. In the exceptional case (see J, Table I), the cut ends of the descending and ascending filaments had not fused together, but had rounded off independently, the two lamellæ being unconnected in the region of the injury. The ventral edges of the lamellæ were irregular, the filaments having irregularly swollen ends, and no food groove had been formed. There was some fusion of the filaments side by side, towards the ventral edge, and they appeared to be in a somewhat degenerating condition. The cut in this instance was made at the extreme posterior end of the gill where the filaments are either without interlamellar junctions, or there is only one to a filament ; such junctions as existed were evidently in the piece of gill which sloughed off following the cut, and there was therefore nothing to prevent the remaining parts of the lamellæ from separating ; the non-fusion of their cut ends was apparently due to this cause.

Mussels, which have been found with the ventral edges of the gills in a jagged condition, have been noticed to have continuous, though irregular, ventral food grooves, and offer additional evidence that a groove is practically always formed at the cut edge. An example of regeneration of a food groove following natural injury is sketched in Figure 18, B, of a previous paper on abnormal gills in this journal (**2**, p. 512).

It was found that two secondary folds bearing food grooves were present on the descending lamella of the left outer gill of the experimental Mussel A, as shown in Figure 2 : some slight abnormality of the filaments occurred in corresponding positions on the ascending lamella. It is perhaps probable that the injuries done to the gill are accountable for these, though there appeared to be no difference in pigmentation between the secondary folds and the old part of the gill. It will be seen that the secondary groove near the ventral edge of the gill, runs from the main food groove in an anterior and dorsal direction, so that the current along its food groove diverges from that of the main groove instead of joining it, as is more usual in secondary grooves on gills (see **1**). On most of the filaments composing the larger secondary

groove there was no reversal of beat of the frontal cilia, but on a very few it occurred for a short stretch near the secondary groove. There appeared to be no change in direction of the ciliary current on the filaments composing the smaller, and more dorsal secondary groove, but the filaments were not examined singly.

Gills (see C, D, E, F and J, Table I) which had been cut with a scalpel obliquely across the filaments—care being taken not to reach the ventral edge of the gill—in some cases the gill being unsupported, and in others supported by the blade of an oyster opener inserted behind the gill, curiously showed not the slightest sign of injury when examined under the microscope at the end of the experiments. The doubt arose, considering the difficulty of working with mussels with the valves only slightly separated, whether any cut had actually been made. Investigation with an opened mussel showed that if the gill was unsupported the frontal epithelium alone was cut, the chitinous skeleton being sufficiently resistant to prevent cutting of the entire filament. Following the cut, the epithelium peeled off away from it, leaving the chitinous skeleton exposed for a stretch of about 1·0 mm. When the gill was supported by the blade of an oyster opener the result varied. In some instances both lamellæ were cut through ; in others only one lamella, though the epithelium of the opposite one was injured ; while in others, though the epithelium of both lamellæ was injured, few if any filaments were actually cut through. In all cases the epithelium peeled off for a short distance on either side of the cut or injury. Thus, beyond the cutting of the frontal epithelium, the extent of the injury inflicted with a scalpel is not exactly determinable. However, in the case of the experimental mussels, it is most probable that at least in some instances the filaments were cut through, and there can be no doubt that then the cut ends fused cleanly ; where the epithelium alone was cut or injured with consequent peeling off for a short distance, it had regenerated.

This part of the experiment was done in an unsuccessful attempt to cause the formation of secondary food grooves ; possibly a tear rather than a clean cut might be more efficacious, but pulling a bent mounted needle, which had been ground to a cutting surface, across the surface of the gill, as was done previously with several mussels, also produced no result. By the latter method the filaments seem to be pulled apart and injured, but not broken.

(*b*) Gill Filaments.

Regeneration of the gill filaments did not invariably occur ; in only one (see A, Table I) out of four surviving experimental mussels, was regeneration unmistakable and of some amount (Figs. 1, 2 and 3). In one area on each of the two left gills of this mussel (Figs. 2 and 3) the piece regenerated

was as great or slightly greater in amount than that lost; the maximum length of filament regenerated, 7·3 mm., occurred in a deep narrow area on the left inner gill (Fig. 3), where, however, regeneration was not complete. The rate of regeneration in these gills would appear to have been rather greater than on the right inner gill of the same mussel (Fig. 1), where the greatest length of filament removed was about 5·4 mm., while the greatest length regenerated was about 3·2 mm.; it may be that the shape of the piece severed from the gill has some bearing on this, a narrow deep gap being more rapidly filled in than a broad shallow one.

In two mussels (B and J, Table I) some slight amount of regeneration appeared to have taken place, but it was difficult to distinguish, as owing to the very pale tint of the gills there was no appreciable difference in

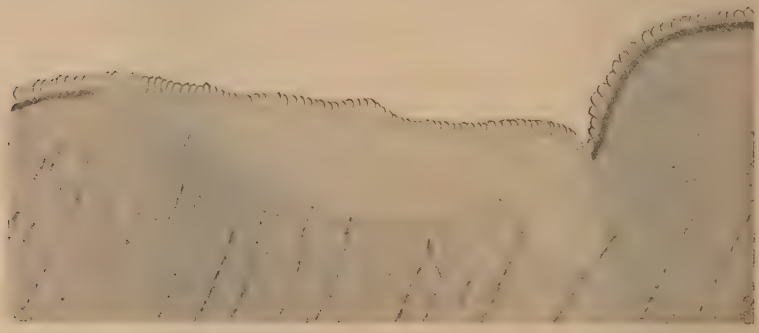

Fig. 1.—Mussel A. Regenerated area on right inner gill; ascending lamella sketched. An attempt has been made to indicate the difference in degree of pigmentation of the old and new parts. The dark line underlying the food groove in the original part of the gill, represents the line of orange or brown pigment normally found in this position; the dark oblique lines represent interlamellar junctions; the dotted wavy horizontal lines, ciliated discs. It will be noticed that a few filaments bordering on the injured area anteriorly, that is at the deepest part of the cut, appear either to have not shared in the growth of the gill, or become reduced in length. (Anterior is on the right.) × ca. 6¼.

degree of pigmentation of the old and new regions. It was judged to have occurred partly on a comparison of the size of the pieces removed with the size of the gaps left in the gills at the end of the experiment, and partly on a consideration of the form of the filaments.

At the junction of the old and new part of the gill there is generally a slight bend in the filaments (Fig. 4). In some cases (see Fig. 5) there may be lateral fusion of the cut ends of some of the filaments so that two or three will grow forward as one, and as a result there are fewer in the new part than in the old. The ventral continuation of fused filaments is shown by sections to be single in structure as well as in appearance (Fig. 6, p. 562), as might be expected if this part were due to new growth; if it were due to fusion side by side of existing filaments this would in all probability be

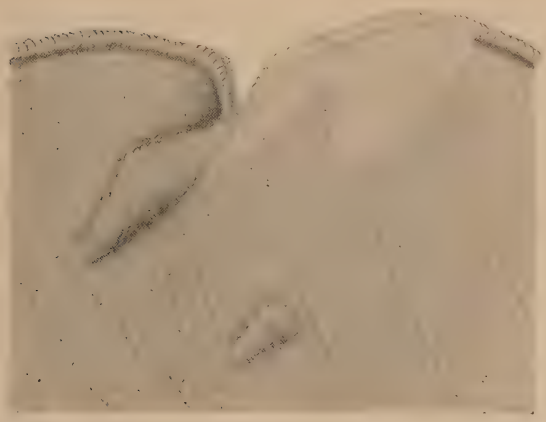

FIG. 2.—Mussel A. Regenerated areas on left outer gill; descending lamella sketched. Two secondary folds with food grooves are shown. (Anterior is on the left.) × ca. $6\frac{1}{4}$.

FIG. 3.—Mussel A. Regenerated areas on left inner gill; ascending lamella sketched. (Anterior is on the left.) × ca. $6\frac{1}{4}$.

apparent in duplication of the chitinous supports in the fused part (see **2**, p. 507). This lateral fusion of the cut ends of certain of the filaments, with forward growth as a single filament, resulted in an irregular number in the descending and ascending lamellæ, and as a consequence, along the ventral edge of the gill instances occurred of the fusion of the ventral ends of two filaments in one lamella, with the ventral end of one in the opposite lamella.

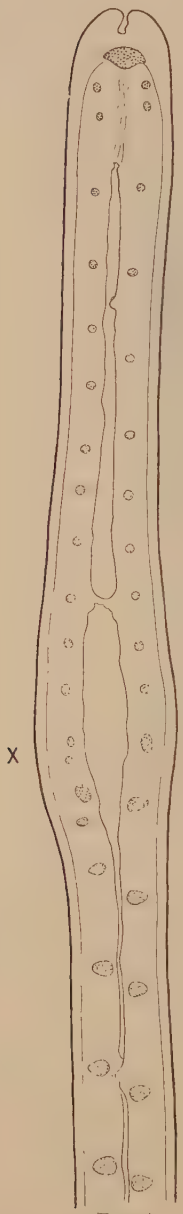

Fig. 4.

But the ease with which slight regeneration can be detected depends largely on the depth of pigmentation of the original part of the gill. The characteristic appearance of the normal gill—when looked at as a whole—with (*a*) the line of orange or brown pigment beneath the main ventral food groove; (*b*) the more opaque, sometimes darker, wavy lines running longitudinally across the surface of the lamella, due to the presence of ciliated discs; and (*c*) the more opaque, and sometimes darker, oblique lines, due to the presence of interlamellar junctions, is shown in the old part of the gill in the sketches in Figures 1, 2 and 3. These three sets of markings on the gill are more clearly visible in some gills than in others, depending largely on the variation in general depth of pigmentation of the gills, which occurs in different individuals. In two mussels (B and J, in Table I) the gills were so pale in tint that even the regenerated food groove showed up very faintly, though under the microscope the difference in pigmentation could be fairly easily seen. In one mussel (A, in Table I), in which an appreciable amount of regeneration had occurred, the regenerated areas were obvious at a glance owing to the difference in intensity of pigmentation between them and the old part of the gill (Figs. 1, 2 and 3). The newly formed parts were practically unpigmented, and the dark line of pigment normally present

Fig. 4.—Side view of filament from a region of regeneration. The junction (at ×) between the old and new parts of the filament is marked by a slight bend: the difference in the size of the ciliated discs in the two regions is noticeable, those in the new part being considerably smaller than those in the old part. One interlamellar junction is present in the new region and also the beginning of a second. Bouin's fixative. × ca. 35½.

running parallel with the free ventral edge of the gill was lacking; the pigment granules seen under the microscope in this position being too few to give any appearance of colour. In the case of the right-inner gill of mussel A (Fig. 1) the oblique dark lines on the old part of the gill, due to the presence of interlamellar junctions, were seen to stop abruptly when reaching the regenerated portion. Under low powers of the microscope, or with a lens, the dark wavy lines caused by the presence of the ciliated discs on the filaments were also seen apparently to stop, or alter in character, against the new tissue in all cases of regeneration of the gills of this mussel (Figs. 1, 2 and 3): in the regenerated region

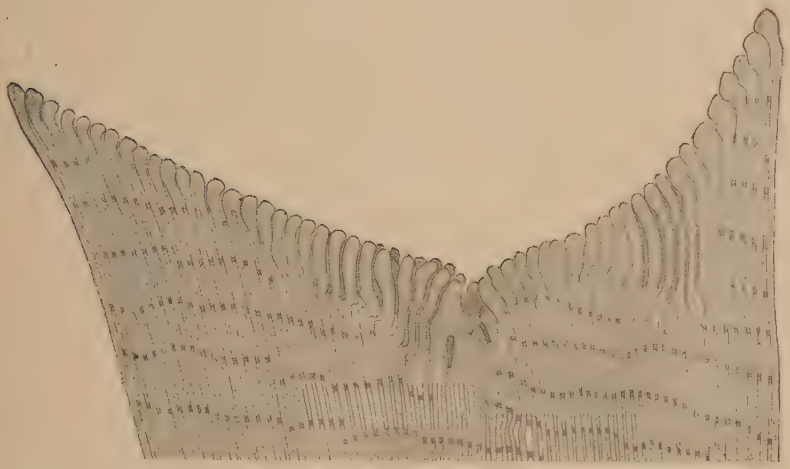

FIG. 5.—Mussel B. Left outer gill, ascending lamella. A small region at the depth of the gap has been sketched, to show the tiny irregular area of regeneration, and the apparent fusion of some of the filaments. In this instance there was no appreciable difference in colour between the old and new parts. (Anterior is on the right.) × ca. 18¼.

these lines, though present, were faint, and difficult to follow. The cause of the difference in intensity of the lines was apparent on examining single gill filaments in side view; in the regenerated part of the gill filament the ciliated discs were on an average about half the size of those in the original part of the filament (see Fig. 4), and were practically destitute of pigment granules. In those instances examined, they also differed in shape, being more or less circular, while those in the old part were somewhat triangular. Single filaments stripped from one part of a gill (preserved in formalin) where regeneration had occurred, showed the mucus cells, which were plentiful on the abfrontal face in the old part, to be absent, or not visible in a total unstained preparation, in the new part. This, however, is probably a point of little importance as the condition of the mucus glands is

likely to vary in different parts of an uninjured filament, and at different times. Microscopic transverse sections through the old and regenerated parts of the gill showed no appreciable difference in the development of the chitinous supports. The difference in pigmentation between the old and

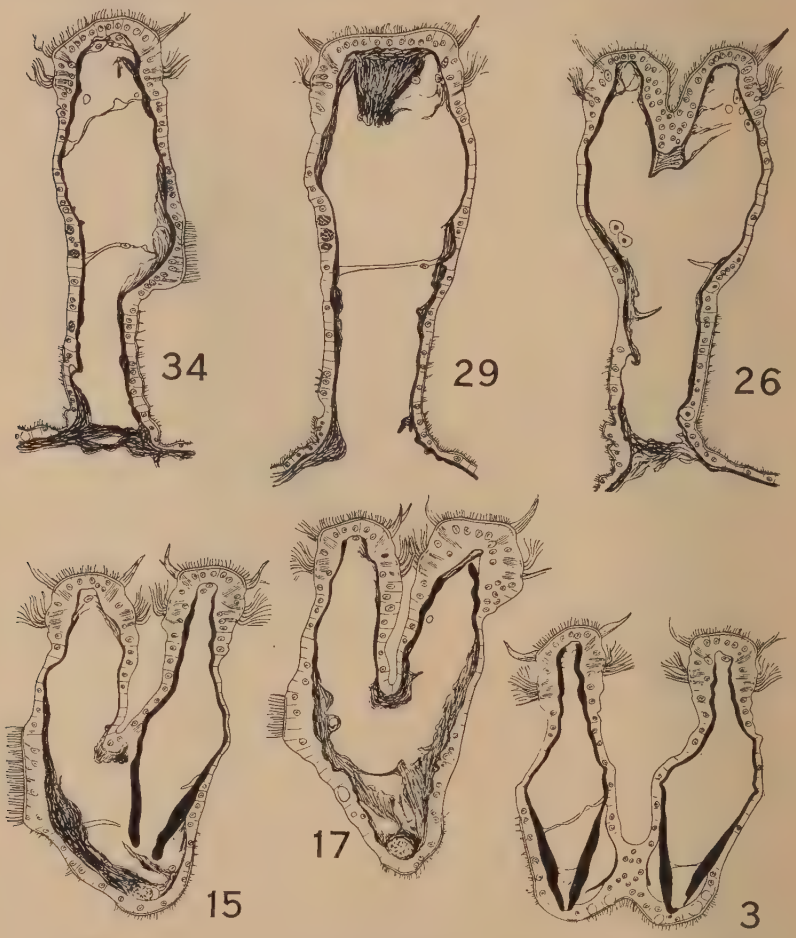

FIG. 6.—Mussel B. Left outer gill, ascending lamella. Transverse sections from a series passing through the junction of the two fused filaments on the extreme right of Fig. 5, to show the change undergone by the chitinous supports and the simple character of the ventral continuation of the filaments. Some slight fusion of the two filaments was evident in the two sections preceding the most dorsal one sketched, namely, 3. The sections are numbered according to their position in the series, so that the number of intervening sections may be known. In the 22nd and following sections (see 26, 29, 34) the filament was connected with several, more posterior, filaments and also with a group in the opposite lamella. The pale staining chitin is indicated by shading. Bouin's fixative; Mallory's Triple Stain. Sections ca. 8μ thick. $\times$ 270.

new food grooves was clearly visible in sections passing from one to the other : these were cut a day or two after fixation and the pigment granules had retained much of their original yellow colour.

Interlamellar junctions, to the number of two, have been observed in the regenerated part of some of the few filaments examined singly : they were finer than those in the original part of the filament. In the new part, some at least of the interlamellar junctions appear to be formed by outgrowth from the abfrontal face of one filament (Fig. 7), three cases being noticed of small outgrowths in this position ; two of these, of which note was especially taken, were from the ascending filament and were respectively about 1·2 and 2·59 mm. from the ventral edge of the gill. This is

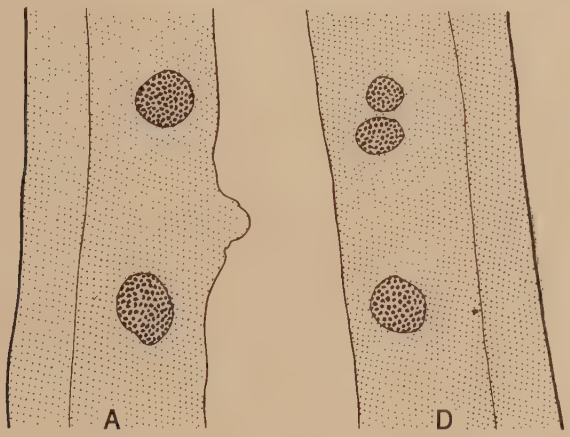

Fig. 7.—Sketch showing origin of interlamellar junction on abfrontal face of ascending part of regenerated filament. The small outgrowth was about 2·59 mm. from the ventral edge of the gill. Bouin's fixative. D and A, descending and ascending filaments respectively. × 140.

of interest in relation to the method of formation of these junctions in the normal development of the gill. According to Rice, they arise through the perforation of a short interlamellar septum, present between the ventral ends of the descending and ascending filaments (see **12**, Fig. 8, p. 73), and such as is found much more fully developed in Modiolus. This he refers to as the Modiolus stage in the development of the filaments of Mytilus.

In the regenerated areas, as shown by sections through the regenerated part of the left outer gill of Mussel B (Fig. 5), much interfilamentar as well as interlamellar connection may occur, in part obliterating the interlamellar space. The condition of the filaments shown in Figure 8 was seen in part of the regenerated area, the particular section sketched being

across some of the normal-looking filaments to the right of the median area of fusion, and between the two most ventral rows of ciliated discs seen in Figure 5. In some parts a similar condition of groups of filaments existed through much of their regenerated length (ca 1·0 mm.).

In a certain number of mussels slanting cuts were made, with scissors, from the ventral edge of the gill in a dorsal and anterior direction, in this way separating a triangular piece from organic connection with the gill, but without removing the piece, which remained connected merely by the

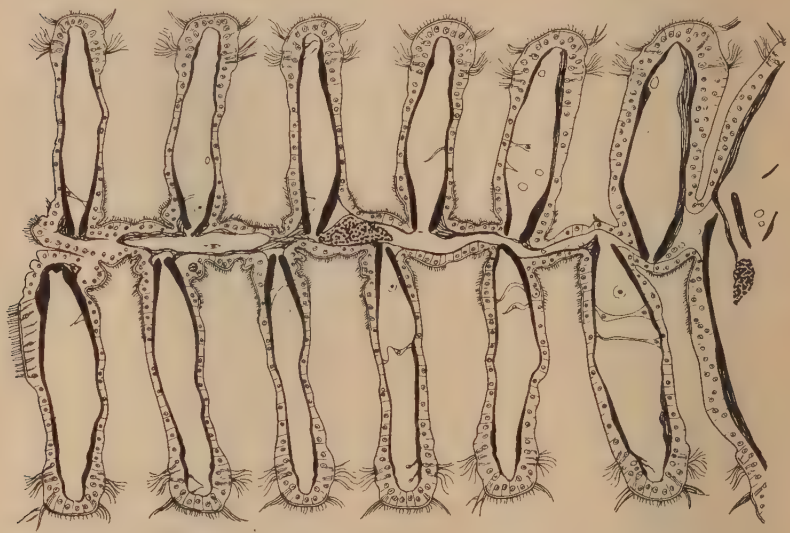

Fig. 8.—Mussel B, left outer gill. Transverse section through part of new area sketched in Fig. 5, showing the filaments in the two lamellæ connected by a median band of tissue. A mass of pigment is present at one place within this median connection. This section is across some of the normal-looking filaments to the right of the median area of fusion, and between the two most ventral rows of ciliated discs, seen in the sketch. Bouin's fixative; Mallory's Triple Stain. Sections ca. 8μ thick. × 200.

ciliated discs on the most anterior and longest length of filament. In the four gills treated in this way (mussels B and J with one gill in each, and mussel A with two gills cut), the pieces had more or less completely degenerated and fallen off. In one instance (left inner gill of B), it appeared that the cut ends of some few disconnected filaments at the depth of the cut (the longest pieces separated) had joined up again, but somewhat irregularly, the ventral ends of two joining the dorsal end of one and vice versa: in this case also, while most of the cut ends of filaments had sloughed off, there were five or so short lengths of filaments separated dorsally from the main part of the gill by a tiny space, but attached anteriorly

and posteriorly; but it is most probable that these filaments were in connexion with each other and the rest of the gill by some organic junctions.

In a previous experiment in June, 1929 (see **1**, p. 961), some organically separated ends of filaments were still connected, after 112 days, with each other and with the main part of the gill merely by their ciliated discs. It, therefore, appears that though such cut pieces of filament may persist for a certain time, after a longer period they are very liable to slough off.

In one mussel (A, in Table I) the two left gills were cut in the manner described above, and regeneration occurred, new tissue partly or entirely filling up the triangular gaps (see Figs. 2, 3).

It may be noted that even after preservation in formalin, or in Bouin's picro-formol for rather more than three months, regenerated areas were recognisable owing to their greater translucence.

DISCUSSION AND SUMMARY.

Experiments have shown that the gill of Mytilus is capable of regeneration, and that this may occur in less than eight months. It may be confined to the formation of a food grove at the cut edge of the gill, without appreciable regeneration in length of the gill filaments. Regeneration of a food groove appears always to occur at the cut edge, if the ends of the descending and ascending filaments are able to touch and so to fuse. On the other hand, regeneration of gill filaments does not seem to occur invariably, and when it does the rate is slow, at least under experimental conditions and in mussels of a length of about 7·0 to 8·0 cm., such as were used for the experiments: it is possible that regeneration would occur more surely and rapidly in young mussels, but owing to the thinness of the shell they would be more difficult to wedge open without fracturing. Coulthard (**6**, p. 136), however, says that "The rate of growth is independent of size in the mussel, being apparently influenced only by the environment." Perhaps the lack of an abundant food supply under the conditions of the experiments should be taken into consideration, though it is well known that in general the amount of food available to an animal has little influence on regeneration (**9**, p. 27). The salinity of the water in general circulation is about 36–37‰, that is, higher than normal sea-water, which is about 35‰, and would be considerably higher than the optimum salinity for growth (see Flattely and Walton, **7**, p. 81). This may also possibly have a retarding effect on the initiation of regeneration and the rate.

It is obscure why regeneration does not always occur; so far as could be judged there was no difference in the method of making the cuts, there was little difference in the size and therefore the age of the mussels used,

the cuts were made in approximately the same position on the gill (somewhere between the posterior and middle third of the gill, this position being chosen simply because it was the easiest to reach), and at the end of the experiments—none of which was terminated before the end of eight months—there was little difference in the condition of the various mussels.

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The Natural History of *Bulla hydatis* Linn.

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With one Figure in the Text.

Bulla hydatis is a mollusc of relatively rare occurrence in the Plymouth district, although fairly common across the Channel at Roscoff. Intermittent dredging and collecting in the Salcombe estuary over a period of about five years, and some rearing experiments carried out in the department of zoology, University College, London, have afforded some insight into its apparently peculiar distribution and also a few facts of certain general interest.

This tectibranch mollusc was recorded during the survey of the Salcombe estuary by the staff of the Plymouth Laboratory in 1902, but it was found only on a spit of sandy gravel on the south shore of the Salstone. Apart from this, it apparently has not been seen except for a few specimens found in the Yealm estuary and one from Jennycliff bay at about the same period. During the succeeding twenty-odd years no record of Bulla was made at all, although there is some evidence that its spawn has been seen though not recognised.

It is accordingly of greater interest that it has been found on almost every visit, winter and summer, made to the Salstone since 1924 ; and it was discovered on each occasion only on the sand spit mentioned above, and below the low-water spring-tide level.

Spawning occurs in June, and a few weeks before that of Oscanius, which is commonly found on the same grounds. The spawn is shown in Figure 1, A, and is usually attached to weed, sometimes to stones, near the datum line. The eggs are approximately 0·2 mm. diameter and two are contained within one capsule ; the capsules are arranged in rows along the shorter axis of the jelly (Fig. 1, A and B).

Hatching occurs after two or three weeks when the veliger larvæ have been actively swimming within the capsules for a considerable period. On hatching great variability is seen in their behaviour, some swimming as veligers for a few days although keeping near the bottom, others settling at once to crawl for the rest of their existence. In other words,

hatching occurs just as the veliger phase is coming to a close, and slight differences in time of hatching may produce great differences in degree of dispersion. However, a large proportion of larvæ are sure to settle immediately, and in this fact lies the explanation of the continuous

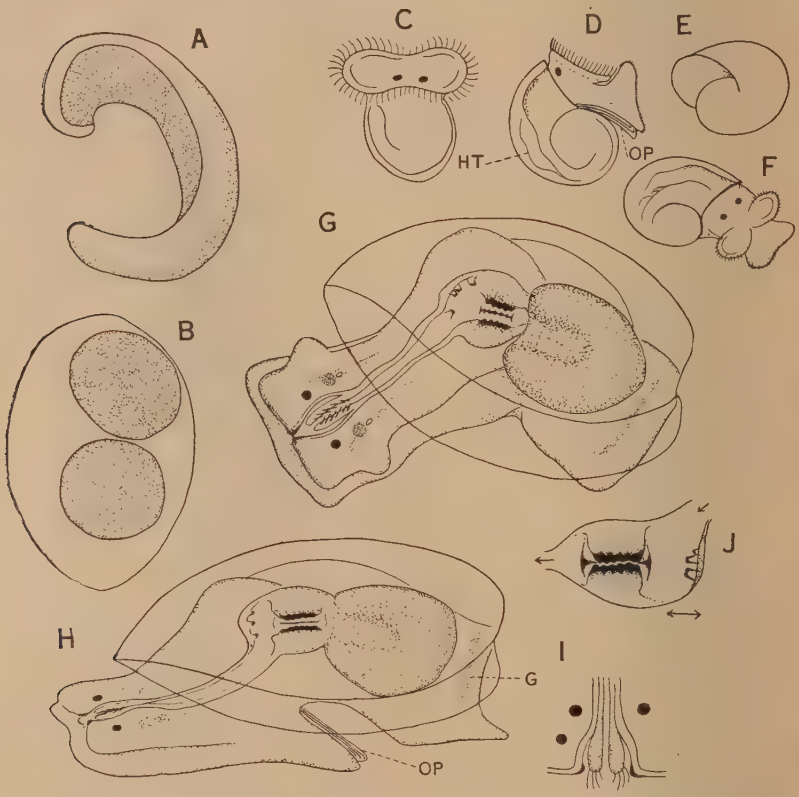

FIG. 1.—A, spawn of *Bulla hydatis* Linn., about natural size. B, developing eggs within isolated capsule ; capsules are arranged in rows as shown in A. C, D, dorsal and side views of veliger larva at hatching stage. E, stage at which large proportion hatch, when velum somewhat resorbed and larva crawls. F, empty shell of the newly hatched larva. G, H, post-larval forms about two months old, showing gastric mill, larval feeding mechanism, and elongation of shell aperture. I, larval "grab" extended (shown retracted in G), also chitinous lips. J, larger view of gastric mill showing the three teeth, the stoking plates and their range of movement.

occupation of a small sand spit for a recorded period of about thirty years. The apparent absence elsewhere along the south-west coast of England may be due to one of two reasons. The free-swimming veliger phase may be too short for the larvæ to be carried to any other feeding grounds, or, it may occur in most southern estuaries of high salinity

without having been found. This last is quite possible, for Bulla apparently lives close to the low-water line, spends much of its time burrowing in the muddy sand, and is to be found much more readily by touch than by sight ; moreover, but little dredging has been carried out in other estuaries in such very shallow water. Its presence in other districts accordingly is more likely to be indicated by the discovery of the yellow or brownish spawn.

A number of spawn jellies were kept in small aquaria and the hatched larvæ reared until a few, after fifteen months, were about 15 mm. long or about one-third adult size. The chief cause of mortality was the tendency to climb above the water level and dry up before regaining paradise. Frequently a tank was considered to be destitute of survivors when, as time proved, many were ploughing their way beneath the sand, often remaining out of sight for a week or two. The only food obtained by these forms was the sheet of diatoms and algæ encrusting the walls of the aquaria, and these small individuals and also adults seemed to scrape the sides with the radula in a manner quite indiscriminate. Adults opened on capture contained only remains of hydroids and algæ.

Certain observations of some interest were made upon post-larval stages and are illustrated in Figure 1.

Most larvæ upon hatching are able alternatively to swim as veligers or crawl as gastropods ; the shell at this time is a simple coil, the opening of which can be closed by the operculum carried on the posterior border of the foot. As post-larval growth proceeds the long ciliated lobes of the velum are resorbed, and the body and shell become much elongated so that the operculum no longer is adapted to the shell opening.

Shortly after the crawling existence commences and until the individual is several millimetres in length, feeding is accomplished by a temporary mechanism. This exists probably because a true radula cannot be encompassed within so small an organism. It is illustrated in Figure 1 and is composed of setæ that project divergently when the buccal mass is extruded ; when retracted the setæ close together and are withdrawn, the whole action being that of a grab.

Peristaltic movements convey the food from the buccal cavity to the gastric mill. The exact time and details of the change over to the adult radula mechanism were not determined. The activity of the gastric mill, however, could be seen quite plainly through the transparent tissues. The mill itself consists of three massive teeth, the grinding surfaces of which form an angle of 120 degrees to each other and bear ridges ; the whole tooth on removal from the muscle in which it is embedded might at first sight be mistaken for a small specimen of Chiton. These three teeth fit together and complete a circle ; they have two movements, a simple withdrawal from their common centre alternating with their

mutual approach, and a rocking movement along their long axes after they have approached one another as closely as possible. If there were food in position the one movement would act upon it as a vice, the other as a grinder. If a cross section of the mill be visualised, three radii will be seen corresponding to the spaces between the teeth. In front of the mill is a small crop, the anterior wall of which bears three groups of hard plate-like projections. If this wall is pushed toward the mill the three projections fit into the three radii mentioned above ; consequently any food contained within the crop would get pushed accurately into position in the mill. That this does happen is shown by the fact that the anterior wall of the crop pulsates regularly once per second whether or not any food is present, and the wall with the projections it bears acts as a perfect automatic stoking apparatus. Figure 1, G, I, and J, illustrates the above description.

In conclusion a word may be said concerning the significance of hatching enzymes, especially among invertebrates where larval stages are so important.

It has been demonstrated that among the lower vertebrates (Wintrebert, 1926) and the ascidians (Berrill, 1929) proteolytic enzymes are mainly responsible for the process of hatching, and that among the ascidians at least the normal activity of the enzyme is very easily disturbed. Similar enzymes for hatching most certainly occur in many molluscs, in particular in Cephalopods such as Loligo and Sepiola, and in Opisthobranchs including Bulla.

The process seems to be the same in these last two groups—a progressive softening of the jelly mass throughout the period of development, and a digestion of the capsule membrane at the end of that time.

Not only are there differences among the gastropods in the time at which hatching occurs but also in the size of the egg, i.e. in the amount of yolk or reserve food it contains. Such yolk may be confined for the most part to the endoderm and in consequence be drawn upon by the whole organism as is the yolk sac in the case of vertebrate embryos ; alternatively the yolk may be evenly distributed through all cells ; in either case the embryo or larva, compared to embryos developing from smaller eggs, may reach a more advanced stage of development before having to obtain food from without.

The smaller the eggs the sooner they must hatch as larvæ and fend for themselves. At the same time they become subject to a high rate of elimination earlier in life, for until hatching occurs they are relatively safe, a degree of safety comparable to that produced by viviparity in other forms. Moreover, larvæ will be eliminated after hatching at the same rate whether they be large or small. Accordingly any increase in egg size, which necessarily implies a decrease in egg number, will only be of survival

value if the protected phase of development be prolonged—in other words, if hatching be delayed.

The eggs of most gastropods which possess a prolonged free-swimming veliger phase (possibly of several weeks) have egg volumes much less than that of Bulla (and of the allied form, Scaphander). Since there is no evidence nor suggestion that reduction of yolk has occurred in the group Opisthobranchiata, this smaller egg size may be considered primitive, and accordingly the postponement of hatching in Bulla until the veliger phase is almost over must be considered a direct result of yolk accumulation within the egg.

How such retardation of the hatching process is effected is not known. It is possible that the veliger stage is reached when the embryo consists of a certain number of cells, that is, various sized veligers are composed of approximately the same number of cells but vary in cell size, while hatching enzymes are liberated and become effective when the cells responsible are reduced by successive cleavages to a certain volume. In other words, the larger the egg the larger will be the veliger, but the longer will it be before the hatching enzyme is formed.

In the case of Bulla the importance of the enzyme is obvious. A slight acceleration of its action would result in the liberation of all the embryos as free-swimming larvæ ; they would be dispersed over a very wide area but might fail to settle on the parental feeding ground. On the other hand, a slight retardation of its action would confine the veliger phase entirely within the egg capsules, the population of the parental grounds would be maintained and possibly become too dense, and there would be little chance of colonizing new areas ; and any changes tending to eliminate the veliger phase from development structurally as well as functionally could take place with impunity.

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Notes on Cyanea Caught in the Ring-trawl in the Plymouth Area during the Years 1925 to 1930.

By

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During the researches on the vertical distribution of plankton and on the seasonal abundance of young fish, a record was kept of all *Cyanea lamarcki* Péron and Lesueur* occurring in the catches of the 2-metre stramin ring-trawl. This was primarily for correlation with the abundance of such fish as the young whiting, *Gadus merlangus*, which at certain stages in their development shelter under the umbrella of the medusa. Since it is only with the larger Cyanea that this association of the young whiting exists it was necessary to keep a record of the sizes of the Cyanea occurring in the catches. These were only rough measurements and denote the diameter of the flattened disc of the medusa usually to the nearest half-inch.

Records of the sizes of Cyanea species occurring in other latitudes have been published from time to time, but the writer can find no record of the sizes occurring at Plymouth and it seemed desirable to publish these observations.

In Table III at the end of this paper are given all the numbers and measurements of Cyanea caught in the ring-trawl. All the data for the hauls in question have been given in previously published papers in this journal dealing with the vertical distribution of plankton or the abundance of young fish. It is only necessary to state here that on all dates in the years 1925 and 1926 the numbers given are the total numbers taken in five or usually six ten-minute hauls, each haul being taken at a different depth. From 1927 onwards the collections on each day were half-hour oblique hauls.

A study of these records shows that Cyanea was only taken in the months of April, May, June, and July. Not a single specimen was caught in any other month in the year. In Table I are summarised the results for all years showing the numbers at the different sizes occurring in the months April to July.

* The validity of *Cyanea lamarcki* as a distinct species seems still to be in doubt (*vide* Bigelow, **1**, p. 357, and Krumback, **6**, p. 24), but I have here followed the nomenclature of the Plymouth Fauna List. In my previous publications I have followed Mayer (**7**, p. 596) in regarding *lamarcki* as a variety of *C. capillata*. Vanhöffen (**8**, p. 53) regarded *C. lamarcki* as a distinct species.

TABLE I.

Diameter of disc in inches.

	<2″	2″	2½″	3″	3½″	4″	4½″	5″	5½″	6″	6½″	7″	7½″
April	118	3	–	2	–	–	–	–	–	–	–	–	–
May	45	6	6	7	–	4	–	–	–	–	–	–	–
June	21	11	8	8	1	12	1	2	1	1	–	–	1
July	4	1	1	2	2	3	–	5	–	2	1	–	–

It shows that Cyanea of a very small size, many of about half an inch diameter or less, appear in comparatively large numbers in April, the largest taken in that month being only 3 inches in diameter. The numbers of small medusæ of under two inches get steadily lower until in July only 4 were taken during the five years in which collections were made in that month. At the same time the numbers of large specimens increase. It appears that 5 to 6 inches in diameter is the usual limit of size reached, one only of 7½ inches having been taken.

In Table II are given the average catches per half-hour oblique haul for each of the months April, May, June, and July for the years 1925 to 1930. (These figures have been calculated as in Journ. Mar. Biol. Assoc., N.S., Vol. XVI, No. 3, p. 707.)

TABLE II.

	1925	1926	1927	1928	1929	1930
April . .	0·0	11·3	0·0	0·0	2·3	1·6
May . .	0·6	4·1	2·0	—	9·8	1·3
June . .	0·4	1·3	1·7	—	10·7	1·3
July . .	0·8	0·0	0·8	—	1·4	1·0

This shows that in 1926 and 1929 the Cyanea were distinctly more abundant than in the other years.

The size of these blue Cyanea is generally given as from 200–300 mm. diameter (Vanhöffen, **8**, p. 53). Haeckel (**5**, p. 530) gives 100–200 mm., and rarely 300 mm. These measurements are slightly higher than those given in Table I, for while 200 mm. is 7·9 inches, 300 mm. is 11·8 inches. It is possible that these larger sizes occur here but are not caught in the ring-trawl; at any rate, they cannot be very common.

Miss Delap (**2**, p. 21) reared *Cyanea lamarcki* in an aquarium. Her first ephyræ were liberated by March 12th, and by April 15th the largest was 10 mm. in diameter. The latter would correspond to about the size at which they first appeared in April in the ring-trawl catches. Under aquarium conditions the largest size she succeeded in rearing the Cyanea to was only 80 mm. (3·2 inches) by June 10th. In 1901 M. and C. Delap record in Valencia Harbour "A good many seen in June from two to five inches in diameter" (**3**, p. 15), and for 1902 they say, "A considerable number seen on May 8th, from ¾-inch to double that size, all young stages" (**4**, p. 13).

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TABLE III.

Numbers and Sizes of Cyanea taken in the 2-metre stramin Ring-trawl Collections at Plymouth from 1925 to 1930.

1925.

April	2nd, 8th, 22nd, 29th:	none.
May	19th (i.)	1 (" small ").
„	19th (ii.)	1 („).
June	4th (i.)	—
„	4th (ii.)	—
„	17th (i.)	½″, 1: 2″, 1.
„	17th (ii.)	—
„	17th (iii.)	3″, 1.
„	18th (i.)	2″, 1: 4″, 1.
„	18th (ii.)	1½″, 1.
„	18th (iii.)	—
„	18th (iv.)	3″, 1: 4″, 1.
„	19th (i.)	—
„	19th (ii.)	2½″, 1.
July	1st (i.)	¾″, 1: 1½″, 1: 3″, 1: 3½″, 1.
„	1st (ii.)	1½″, 1.
„	16th	—
„	29th	1½″, 1.
August	6th	—

1926.

April	9th	½″–1½″, 12: ⅜″–1¼″, 6: under 1″, 6.
„	13th (i.)	¼″–¾″, 5: ¼″–½″, 7: 1″–1¼″, 2: 1–1¾″, 4: 3″, 1.
„	13th (ii.)	½″, 25: ½″–1½″, 20: 1″, 1: 2″, 1.
„	22nd	¼″, 1: ½″, 2: ¾″, 1: 1″–1⅜″, 3: 1″-2″, 4.
„	26th	½″, 2: ¾″, 1: 1¾″, 1: 2″, 1.
May	6th	½″, 1: 1″, 2: 1½″, 1: 3″, 1: 4″, 1.
„	19th	<1″, 3: ½″, 4: 1″, 1: 3″, 1.
June	3rd (i.)	—
„	3rd (ii.)	—
„	3rd (iii.)	1¾″, 1: 2″, 1: 2½″, 1: 4½″, 1: 6″, 1.
„	4th	½″, 1: 2″, 1: 3″, 1: 4″, 4: 5″, 2: 5½″, 1: 7½″, 1.
„	25th	—
„	30th	—
July	6th, 13th, 15th, 26th:	none.
August	4th; Sept. 22nd:	none.

1927.

April	4th, 5th, 20th, 26th:	none.
May	2nd	—
„	9th	1½″, 2: 3″, 1: 4″, 2.
„	16th	2½″, 1: 3″, 2.
„	25th	—
June	2nd	1″, 2: 1½″, 1: 4″, 1.
„	9th	—
„	29th	2″, 1: 4″, 1.
July	8th	4″, 1.
„	12th	2″, 1.
„	21st	—
„	26th	4″, 1.

1927 (*cont.*)

August	4th, 8th, 19th, 26th, 31st:	none.
Sept.	6th, 15th, 19th:	none.
Oct.	4th, 13th, 18th, 24th:	none.
Nov.	1st; Dec. 15th, 21st:	none.

1928.

Jan.	9th, 16th, 26th, 30th:	none.
Feb.	2nd, 20th, 27th:	none.
March	5th, 21st, 30th:	none.
April	4th, 11th, 12th, 23rd:	none.

1929.

April	10th	—
„	19th	¾″, 3: 2″, 1: 3″, 1.
„	23rd	¾″, 2: 1″, 1: 1½″, 1.
„	29th	—
May	6th	½″, 5: 1″, 1: 2″, 1.
„	13th	½″, 6: ¾″, 3: 1″, 5: 1¼″, 2: 2″, 4: 2½″, 1: 2¾″, 1.
„	23rd	—
„	27th	1″, 2: 1½″, 1: 2″, 1: 2½″, 3: 3″, 2: 4″, 1.
June	6th	¾″, 1: 1″, 4: 1½″, 6: 2″, 4: 2½″, 3: 3″, 3: 4″, 2.
„	11th	1″, 1: 1½″, 1: 2″, 2: 2½″, 2: 3″, 2.
„	25th	1½″, 1.
July	3rd	4″, 1: 5″, 2: 6″, 1.
„	9th	—
„	18th	2½″, 1: 3″, 1: 5″, 1.
„	23rd	—
„	30th	—
August	9th, 15th, 22nd, 26th:	none.
Sept.	4th, 6th, 10th, 17th, 24th:	none.
Oct.	3rd, 10th, 16th:	none.

1930.

Feb.	2nd, 12th, 19th, 26th:	none.
March	5th, 12th, 19th, 27th:	none.
April	2nd	—
„	11th	½″, 1.
„	16th	—
„	24th	½″, 5: ¾″, 1.
„	29th	½″, 1.
May	7th	¾″, 1: 1″, 1.
„	15th	½″, 1.
„	22nd	½″, 1.
June	10th	—
„	19th	3½″, 1: 4″, 2.
„	26th	2¼″, 1.
July	4th	—
„	9th	—
„	14th	—
„	23rd	5″, 1: 6″, 1.
„	29th	3½″, 1: 5″, 1: 6½″, 1.
August	8th, 14th, 21st, 28th:	none.

Notes on the Ciliate *Boveria stevensi* Issel from *Galeomma turtoni* Sowerby at Plymouth.

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and

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With 2 Figures in the Text.

The little bivalve mollusc *Galeomma turtoni* is not very common off the coast of South Devon ; but two specimens were brought in while we were working at the Marine Biological Laboratory last August, and when we examined them we found that the branchiæ and mantle cavity were aswarm with a small colourless ciliate.

A formidable list of ciliates from such situations in lamellibranchs and gastropods stands in the records. From *Galeomma turtoni* Sowerby at Naples Issel (1903) described *Boveria stevensi*, and there seems no reason to doubt that the organism we have found is Issel's ciliate, though the specimens in the Plymouth mollusc are rather larger than those he measured.

We feel justified in publishing the following notes, since Boveria and its allies, the " Thigmotricha " of Chatton and Lwoff, show many features of interest to the protozoologist, and the descriptions of the mouth region in the smaller forms are very inadequate.

The body of our ciliate is 16–36μ long and about 16–20μ broad (the largest measurement Issel gives is $29 \times 17\mu$). In side view it appears curved like an arc ; we shall speak of the convex surface as dorsal and of the more concave as ventral. The maximum thickness is about 15μ. The aboral end, which is directed forwards as the animal swims, is a somewhat pointed lobe.* From the dorsal aspect and in profile, the opposite end appears obliquely truncate : this is the region of greatest breadth and

* De Morgan (1925) and some other observers regard this end in Ancistrum and Boveria as posterior, because the mouth lies at the opposite pole. Maupas (1883) and Cheissin (1931) consider that, as the aboral end is directed foremost, it should be called the anterior end. We prefer to use the terms " oral " and " aboral " to indicate which pole we refer to, without committing ourselves further. It should be noted that the young vorticellid, when liberated from its stalk, swims aboral end foremost ; yet it is customary to speak of the oral end of the stalked stage as " anterior."

thickness. The body does not seem to be very contractile, but preserved ciliates are almost always somewhat shorter and thicker than living ones : the measurements given above were made on preserved material.

The whole surface is covered with longitudinal rows of fine cilia ; there are 10–12 rows on the dorsal side and about the same number on the ventral. Their distribution is best indicated by the lines of basal granules

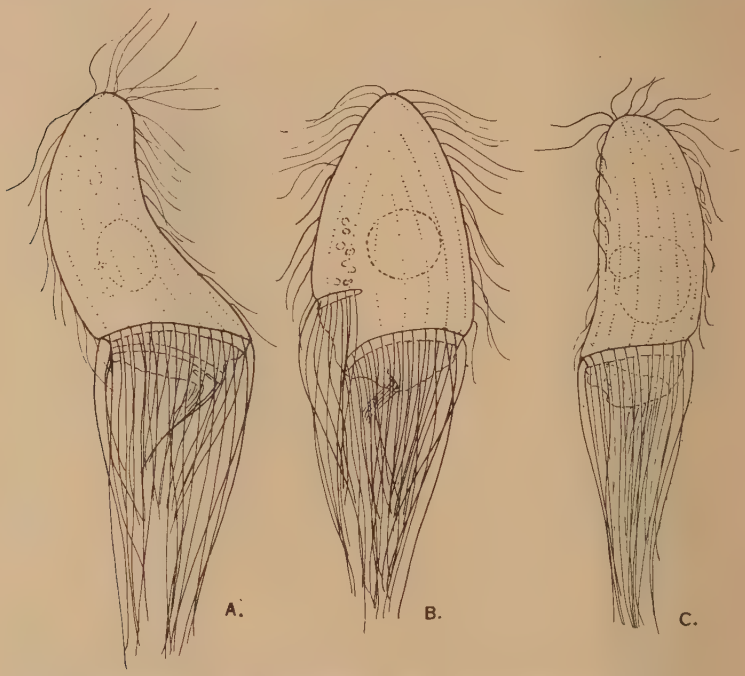

Fig. 1.—Three aspects of the living ciliate. From free-hand sketches. (The number of longitudinal lines of cilia on the body is actually greater. See Fig. 2.)

A. in profile.
B. ventral view.
C. approximately dorsal view.
×1200.

in stained specimens (see Fig. 2). We find that the ventral lines converge and meet one another at an acute angle, a short way behind the aboral end. Issel (1903) does not show this, and Cheissin (1931) suggests (p. 286) that in this respect his new genus Tiarella differs from the species of Boveria hitherto described. The most anterior of these body cilia are longer than the others and tend to stand more erect than do those further back ; just ventral to the aboral end, they are especially active and their movement creates a sort of vortex. These cilia seem to correspond to the

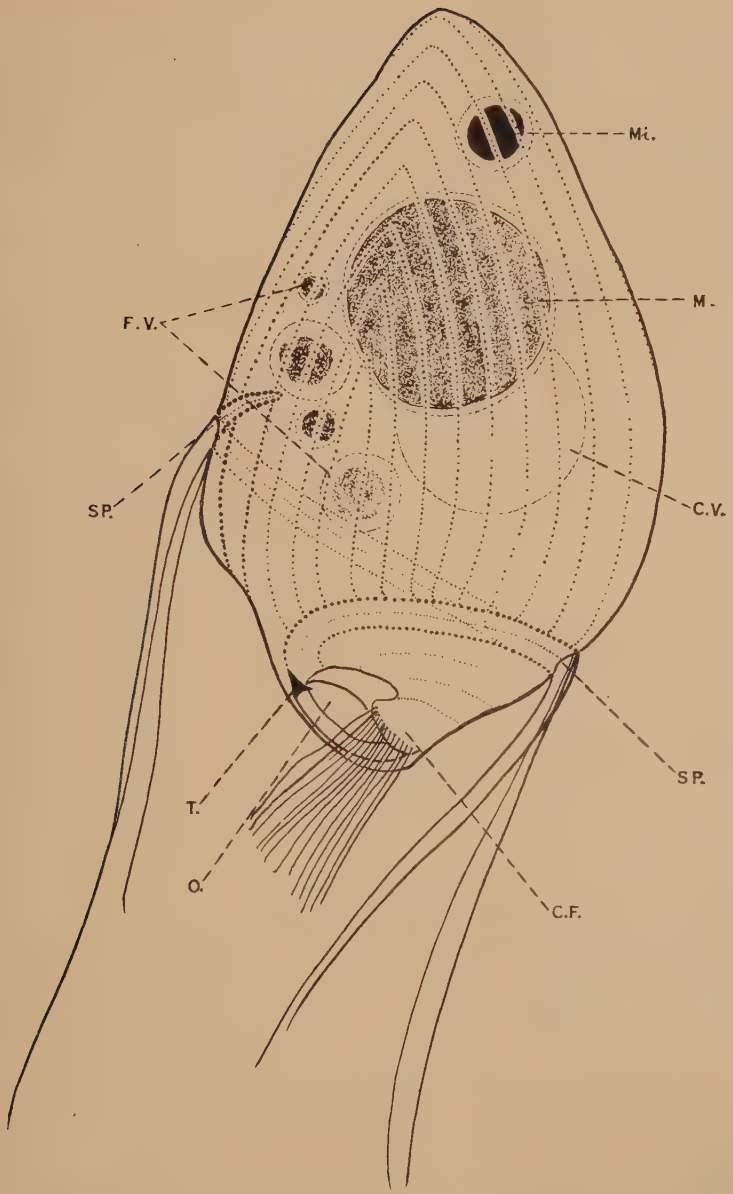

FIG. 2.—Approximately ventral view of a ciliate slightly tilted backwards to show the right-handed spiral in the adoral zone. Drawn with the aid of a camera lucida from an iron-hæmatoxylin preparation. Most of the cilia are omitted.

C.F.	ciliated flap.	M.	meganucleus.
C.V.	contractile vacuole.	O.	mouth.
F.V.	food vacuoles.	SP.	spiral of long cilia.
Mi.	micronucleus.	T.	"tooth."

×2800.

group which, throughout the Ancistridæ and their allies, are said to function as anchors to the substratum. Chatton and Lwoff (1923) make a great point of this " thigmotactic " apparatus, and go so far as to create a special subdivision, Thigmotricha, to include the Ancistridæ, Hypocomidæ, and Sphenophryidæ. They state that the thigmotactic cilia are absent in the genus Boveria, closely allied to the Ancistridæ though that ciliate undoubtedly is. We never saw the organism attach itself by these cilia while alive, but in sections through the gill tissue of Galeomma we have found a few ciliates applied to the epithelium by the ventral patch.

From the truncate oral end arises a tufted veil of very much longer and stouter cilia, which surround the mouth and are undoubtedly concerned with food-capture, though they may also contribute to the swimming movements. Figure 1 gives some idea of the appearance of the living ciliate.

Boveria stevensi swims very rapidly, turning round and round on its long axis as it goes ; sometimes it glides forward, concave side downwards. As it swims, the long peristomial cilia are directed back, and form a sort of tail ; but every now and then the animal pauses, and before darting off again, it raises and lowers the peristomial fringe with a characteristic " grasping " movement.

When the ciliate from Galeomma is alive it is impossible to discover exactly how these adoral cilia are arranged. But stained preparations make the general distribution clear enough, although the details can be determined only after careful comparison of many specimens seen and drawn from all aspects. We find here, as in other Boveridæ, that the peristomial cilia are arranged along a line that follows a right-handed spiral course. There is a double row of these cilia, and, in the slight gutter between them, appears in stained preparations a faint line of minute siderophilous granules from which we could discover no cilia arising.

The cilia in the posterior row of the spiral band are slightly more delicate than those in the line in front ; in the immediate neighbourhood of the slit-like mouth they are finer still and a good deal shorter. To one side of the mouth projects a small, pointed flap, and the cilia bordering this move backwards and forwards as one group and very energetically ; undoubtedly their duty is to drive food into the slit, though we did not actually observe the entry of any particles. Some specimens contained many vacuoles with food-balls, consisting apparently of bacteria. The mouth margin is further complicated by the presence of a minute siderophilous " tooth " arising near the base of the flap from the more anterior of the two lines of adoral cilia. The relations of these parts to one another is shown in Fig. 2. It seems worth while recording these details, since Issel found the organism too small for him to trace the fate of the spiral

in the mouth region—" ma di quest' ultimo tratto, stante la piccolezza del materiale, non sono riuscito a formi un concetto ben sciuro " (Issel).

Dorsal to the meganucleus and towards the posterior end of the body is a large vacuole, which presumably is a contractile vacuole, though we did not observe any pulsation.

The resting nucleus is circular or ovoid in outline and measures about 8–10μ in greatest diameter. It lies about the middle of the thickest portion of the body, and towards the ventral side. In stained specimens its margin is often slightly lobed ; the interior is granular and densely staining. The micronucleus is small and spherical, 3–4μ in diameter, and lies some way anterior to the meganucleus.

The great majority of the ciliates measure about $35 \times 20\mu$. But we have found some that were only half as long, and this suggests that there may have been recent transverse fission. Hoping to find some clue to the mode of origin of the spiral in the daughter organisms, we searched our preparations for dividing ciliates. Many of the larger ones showed signs that their nuclei were initiating the division. The meganucleus in these individuals had increased in diameter (14μ) and had lost much of its staining property ; the micronucleus was either bean-shaped, diplococcus-like, or in two lobes connected by a short, pale-staining band. We failed to find any later stages in the division process. Stevens (1904) states that in *Boveria subcylindrica* fission is oblique ; the peristome and contractile vacuole disappear and are formed anew in the daughter animals ; " the new peristomial cilia appear on the side as a straight band which gradually assumes a terminal position, beginning to coil at the distal end."

A word as to the systematic position of Boveria. *Boveria stevensi* Issel seems to be distinguished from *B. subcylindrica* Stevens mainly by the fact that its peristomial line of cilia runs much more obliquely to the long axis of the body and that these cilia are not of such uniform length. In these respects *B. teredinidi* Nelson more closely resembles the type-species. To include the species of Boveria, Pickard (1927) formed the family Boveridæ, of equal value with the Ancistridæ.

The earlier observers regarded Ancistrum and all its near allies as holotrichous ciliates, related to Pleuronema. Chatton and Lwoff (1922 and 1923) still consider the Ancistridæ as " specialised holotrichans." Stevens' discovery of the adoral spiral in Boveria decided her to remove that genus to the Heterotricha ; Pickard (1927) created a new family, Boveridæ, for its inclusion. Cheissin's new genus Tiarella also seems to belong to that family. But the Ancistridæ and the Boveridæ have so much in common that, if the Boveridæ are to be regarded as heterotrichous, so also must the Ancistridæ.

But is Boveria a true heterotrichan ? All observers have described it as

having a *right*-handed spiral of peristomial cilia—a feature characteristic of the Peritricha (with the exception of Lichnophora and its allies). All true Heterotricha are supposed to have the peristomial spiral wound sinistrally. On this ground Cheissin (1931) goes so far as to say that the Ancistridæ and Boveridæ should be regarded "wahrscheinlich als Abzweigung der primitiven Peritricha."

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An Amœba from the Intestine of an Ascidian at Plymouth.

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With 3 Figures in the Text.

While working at Plymouth in December, 1928, and in August, 1930, we noticed amœbæ in the intestine of *Phallusia mamillata* (Cuvier) which had been dredged in the Sound. The ten animals examined were all infected. The only previous record we can find of an amœba in such a situation is a short account given by Huxley (1920) of a small form occurring in large quantities in the stomach of *Clavellina lepadiformis* at Naples. The amœbæ we have seen are not like those from Clavellina and they seem to merit some description.

The wall of the intestine was carefully slit open and a drop of the brownish fluid contents was drawn off with a fine pipette and examined on a slide. The movements of the parasite, though sluggish, are sufficiently obvious to enable one to distinguish it easily from epithelial débris and other material in the gut.

This is a small amœba, though larger than the one in Clavellina. When active, it is always longer than broad, and, fully outstretched, measures from 15–30$\mu \times$10–15μ. Its most characteristic feature in the living condition is the tendency to form a wide " sole " of clear, glassy ectoplasm, above which rests the main mass of the body, much as the visceral hump of a snail is supported on the muscular foot.

This ectoplasmic sole protrudes as a broad pseudopodium in front, and posteriorly it tapers to a narrow, finger-like lobe. When we used an oil-immersion lens and racked slightly up and down, so that the amœba wobbled under pressure from the cover-glass, we found that it usually remained firmly tethered to the glass or to the intestinal débris by the posterior lobe, while the rest of the body swung clear and might even roll half over. There is very little movement of translation : the amœba contents itself with emitting the broad anterior pseudopodium, then withdrawing this and forming another slightly to the right or left of the first.

In the otherwise hyaline protoplasm of the pseudopodium there often lies a group of small refringent granules. The hump-like mass above the sole is granular and opaque, and its surface tends to be thrown into wrinkles. As a pseudopodium is forming, a number of little knob-like

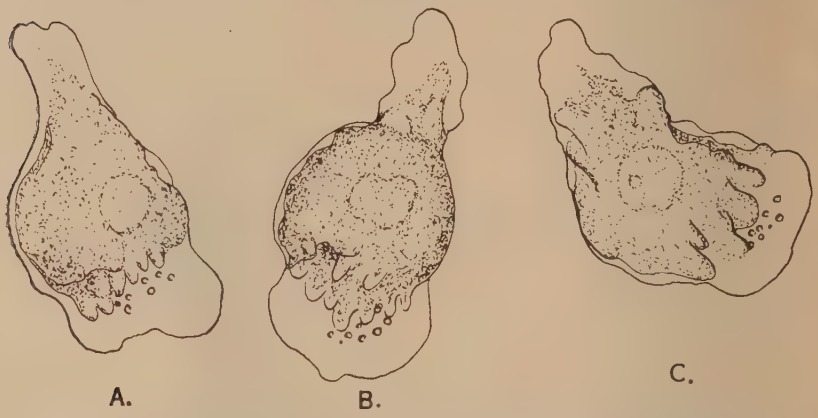

Fig. 1.—A, B, and C. Three sketches of living *Entamœba phallusiæ*, all showing the characteristic "sole" of glassy ectoplasm, with the endoplasmic hump above it. ×2000.

projections of endoplasm appear for a short time between it and the main mass. There is no contractile vacuole. We have not seen the amœba engulf anything, and the granules in the protoplasm do not seem to be food material; it is probable that the organism absorbs its food in a fluid

Fig. 2.—Living cyst of *Entamœba phallusiæ*. ×2000.

state, and the strings and irregular groups of refringent granules that occur in the protoplasm may be excretory matter. The nucleus measures about 4·5μ–5μ in diameter. It is very difficult to detect when the amœba is healthy; but the organism survives removal from its host for only a short time, and as it becomes moribund, the nucleus shows up as a clearer

space, roughly circular in outline but readily deformed by the endoplasmic streaming. Refringent chromatin beads can sometimes be seen just within the membrane, but only very rarely is the karyosome visible in the living nucleus.

We have found a few cysts (Fig. 2). These measure about $21\mu \times 19\mu$. There is a smooth, double-contoured envelope, about $\cdot 8\mu$ thick, and within this the amœba can be seen lying, its surface somewhat folded, and with slight spaces, especially towards one pole, between it and the wall. We have never seen more than one nucleus in a cyst.

When films of the intestinal contents of Phallusia were fixed in Schaudinn's fluid or Brasil's modification of Bouin-Duboscq, the amœba usually contracted very much and became almost circular in outline; only occasionally did we find one with pseudopodium well outstretched.

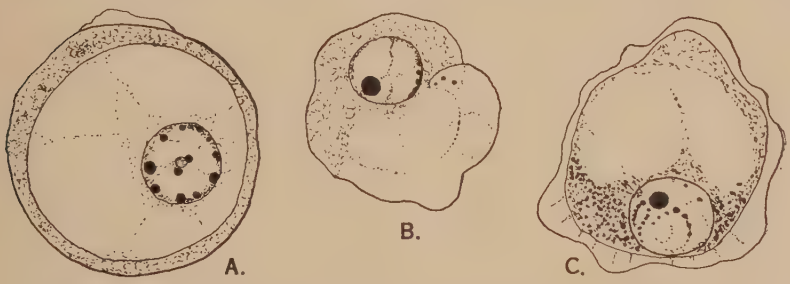

FIG. 3.—A, B, and C. Three amœbæ fixed with Brasil's modification of Bouin-Duboscq and stained with iron hæmatoxylin. The specimens show the retraction of the ectoplasm and the tendency for a large vacuole to appear in the endoplasm. $\times 2500$.

Stained with Heidenhain's iron-hæmatoxylin, such amœbæ show a narrow rim of light-grey homogeneous ectoplasm: the hump invariably appears to contain one relatively enormous vacuole, towards one side of which most of the dark-staining, granular endoplasm is squeezed. Strands of this darker substance criss-cross the vacuolar region, and in one patch, situated almost anywhere near the periphery, lies the nucleus. The refringent inclusions observed in the living organism stain uniformly black and there is no sign of vacuoles around them. The nucleus presents very different appearances according to the amount of its distortion from the spherical and to the angle from which it is viewed. The membrane is delicate; the chromatin is in peripheral beads, and often these are connected together in two or three strings just within the membrane; the karyosome, now very conspicuous but varying greatly in the degree to which it retains the stain, is almost always excentric. We did not find any dividing forms, nor did we see any cysts in the stained preparations.

We have decided to call this amœba *Entamœba phallusiæ* n. sp. The

fact that it has no contractile vacuole does not necessarily suggest that it is a true endoparasite, since the absence of such a structure is the rule in marine amœbæ. But since it seems to feed by imbibing liquid through its whole surface, this indicates that it is rather specially adapted to an endoparasitic life, and it is much more intolerant than are most "facultative" amœbæ of changes in the medium in which it crawls. Its nucleus is not like that of most entamœbæ, if we take the nuclei of *Entamœba histolyica* and *E. coli* as typical; it most closely resembles that of the Entamœba described by Dobell (1914) from the hind-gut of *Lacerta muralis*—a form which he considered quite distinct from *Amœba lacertæ* Hartmann. This amœba from Lacerta, however, was packed with food-balls. Until we know something concerning the behaviour of the nucleus in mitosis it would be rash to speculate further concerning the relationships of *E. phallusiæ* with other parasitic amœbæ.

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Marine Biological Association of the United Kingdom

Report of the Council, 1930.

The Council and Officers.

The Council has great satisfaction in announcing that Lieut.-Col. The Right Hon. Walter Guinness, D.S.O., M.P., formerly Minister of Agriculture and Fisheries, has accepted the office of President in succession to the late Sir Edwin Ray Lankester.

The usual four quarterly meetings of the Council have been held, at which the average attendance has been nineteen. The meetings have been held in the Rooms of the Royal Society, to whom the thanks of the Council are due.

A Committee of nine members of the Council, including the President, visited and inspected the Plymouth Laboratory in March, and a special committee of six members met at Plymouth in September to discuss with the Architect plans for the proposed extension of the buildings.

The Plymouth Laboratory.

The Laboratory buildings and fittings have been maintained in a good state of repair, and the south side of the roof of the main building, the slates of which were working loose, has been overhauled and refastened.

The electric supply for all the laboratories has been redistributed, the necessary light and power cables from the Corporation now being laid underground in place of the previous overhead supply from the Citadel. Power has been supplied to the North Building, with fittings for electric heating in each room.

The engine and circulating pumps for the supply of sea-water to the aquarium and laboratories have worked well throughout the year, the small gas-engine having been fitted with a new liner and piston. During the summer the lower section of the supply pipe from the sea gave out, and a new section has now been fitted.

Proposed Extension of Laboratory Buildings.

In view of the congested condition of the present Library at Plymouth and the increasing number of outside workers who visit the Laboratory, the Council has had under consideration plans for enlarging the buildings. It has been decided to add an additional story to the Allen Building and to use that block entirely for library purposes. In order to make good

the laboratory space which will be lost by so doing, and at the same time to provide some additional working space, it is proposed to extend the present North Building in an easterly direction, following the general lines of the plan of that building. An appeal for subscriptions for these extensions was issued and has met with encouraging success, a sum of £2650 having been promised up to the end of November. With this sum secured the Council has felt justified in proceeding at once with the enlargement of the Allen Building, and its conversion into a Library.

The Ship and Motor-Boat.

The steam drifter *Salpa* returned at the end of January this year from Dartmouth where she was undergoing her Lloyd's full-time survey and reconditioning in Messrs. Philip and Son's yard. She has been certified on Lloyd's register of shipping as A1 for a further period of seven years from March, 1929. She has run satisfactorily throughout the year and kept the increasing number of visiting workers supplied with the material necessary for their research.

The motor-boat *Gammarus* has worked continuously collecting in the Sound, and is in good condition.

The Staff.

Dr. C. M. Yonge took up the post of Physiologist at Plymouth in January on his return from the Great Barrier Reef Expedition. Mr. F. S. Russell, who was also a member of that expedition, has continued to carry out the duties of Administrative Assistant in addition to his scientific research work.

An exchange of staff between the Ministry of Agriculture and Fisheries and the Association took place during the summer. Mr. H. W. Harvey spent some months at the Fishery Experiment Station at Conway, continuing experiments on the rearing of oyster larvæ, and Mr. F. M. Davis of the Lowestoft Laboratory came to Plymouth.

Mr. B. Dawes, who has been in charge of the plaice experiments at Pier Cellars, Cawsand Bay, left in September to take up a post in the Zoology Department of King's College, London. Miss D. Thursby-Pelham of the Lowestoft Laboratory acted as substitute for Mr. Dawes for some weeks during the spring.

Mr. J. S. Colman resigned his student-probationership in September, on appointment as demonstrator in the Zoological Laboratory at Cambridge. Mr. J. E. Smith, of King's College, London, was appointed a Student-Probationer in place of Mr. Colman.

Dr. L. N. H. Cooper, of the University College of North Wales, has been appointed Assistant Chemist in the Physiological Department to assist Dr. Atkins in his work on the minor constituents of sea-water.

Occupation of Tables.

The following investigators have occupied tables at the Plymouth Laboratory during the year :

Prof. Alexandrowicz, Lemberg, Poland (Innervation of the heart of Invertebrates).
Dr. Amirthalingam, Ceylon and London (Central nervous system of Scyllium).
Miss D. Atkins, London (Pinnotheres and Loxosoma).
R. Bassindale, Sheffield (Development of *Balanus balanoides*).
Dr. N. J. Berrill, McGill University, Montreal (Development of Ascidians).
Miss A. Bidder, Cambridge (Digestion tracts of Loligo).
Dr. H. Blaschko, Heidelberg (Experiments on Crustacean nerve-muscle preparations).
Dr. H. Blegvad, Copenhagen (Young Herring and Sprat).
Dr. B. G. Bogorov, Moscow and Murman Coast Laboratory, Russia (Biomass of Calanus).
Miss M. A. Borden, Canada (Respiration and function of hæmoglobin in Planorbis and Arenicola).
M. Burton, London (Sponges).
Prof. H. Graham Cannon, Sheffield (Ostracoda).
Dr. McKeen Cattell, New York (Reflexes in Frogs and work on Crustacean nerve-muscle preparations).
Prof. R. Chambers, New York (Fertilisation and mechanics of cell division. Micro-injections of Plant cells).
Miss M. Cotton, Cambridge (Marine Algæ).
Miss L. Cunningham, Birmingham (Distribution and Feeding Mechanism of Gammarids).
F. M. Davis, Lowestoft (General Fisheries).
W. Fernando, London and India (Embryology of Patella).
Miss E. S. Fogg, London (Plankton).
A. Graham, Sheffield (Enzymes of Pecten).
Dr. A. Grollman, Baltimore (Osmotic pressure of body fluids of marine animals).
J. R. Groome, Oxford (General dissection).
Dr. R. Gurney, Oxford (Copepod larvæ).
L. H. Harrisson, Harrow (General).
G. T. D. Henderson, Bristol (Young Fish).
Miss J. T. Henderson, McGill University, Montreal (Response to stimuli of single muscle fibres).
Miss M. Hetherington, London (Osmotic pressure of body fluids of marine animals).
Prof. A. V. Hill, F.R.S., London (Osmotic pressure of body fluids of marine animals).
W. T. Hillier, Birmingham (Lateral line of fishes).
A. D. Hobson, Edinburgh (Artificial parthenogenesis in Echinoderms and Annelids).
Dr. Karl Höfler, Vienna (Plasmolysis and Permeability of Marine Algæ).
Miss Holt, New York (Cataphoresis of marine eggs).
Dr. and Mrs. Sven Hörstadius, Stockholm (Development of Luidia).
N. H. Howes, London (Pituitary gland of Ray).
A. F. W. Hughes, Cambridge (Respiratory exchange of *Maia squinado*).

K. Hukuda, Japan (Osmotic pressure of body fluids of marine animals).
Prof. J. S. Huxley, London (Differential growth in animals).
Dr. O. G. Ibañez, Madrid (Oceanographical chemistry).
Dr. I. J. Izquierdo, Mexico (Maia heart and water respiratory current).
Dr. R. T. Jackson, U.S.A. (*Echinus esculentus*).
C. C. John, India (Development of Sagitta).
J. A. Kitching, Cambridge (Respiratory exchange of *Maia squinado*).
Dr. M. Knight, Liverpool (Marine Algæ).
Dr. E. Kreps, Leningrad and Murman Coast Laboratory, Russia (Muscular physiology of Invertebrates).
Dr. E. Leloup, Belgium (Hydrozoa).
Dr. E. Lundsgaard, Denmark (Chemistry of Crustacean muscle).
Prof. D. L. Mackinnon, London (Protozoology).
R. M. Margaria, Italy and London (Osmotic pressure of body fluids of marine animals).
N. N. Murti, India (Development of Ophiocoma and Ophiothrix).
Dr. T. G. Ni, Pepin, China (Physiology of glandular tissues).
Dr. E. A. T. Nicol, Edinburgh (Feeding habits in Galathea).
A. G. Nicholls, Australia (Moulting, feeding, breeding and digestion in Ligia).
Dr. K. Oshima, Japan (Hydrography).
G. W. Otter, Cambridge (Gephyrea).
C. F. A. Pantin, Cambridge (Gunda environment).
J. L. Parkinson, London (Osmotic pressure of body fluids of marine animals).
Dr. N. Payne, Pennsylvania, U.S.A. (Hydroid pigments and low temperature effect on Hydroids).
Miss S. Pieh, Munich (Hydrozoa).
A. D. Ritchie, Manchester (Physiology of Pecten muscle).
Dr. P. Rothschild, Heidelberg (Action currents in muscle).
Miss S. S. Ross, Edinburgh (Polyzoa).
Dr. H. N. Roy, London and Calcutta (Parasitic Protozoa).
Dr. Otto Schindler, Vienna (Morphology of young fish).
Dr. E. A. Spaul, London (Pituitary gland of Ray).
Miss F. A. Stanbury, Birmingham University and Plymouth Technical College (Effect of light on growth of diatoms).
Dr. T. A. Stephenson, London (Photography of Anemones).
F. C. Stott, Manchester (Effects of Ions on Ciona heart).
H. H. Sturch, Plymouth (Algæ).
Miss M. A. Tazelaar, London (Parasitic Protozoa).
Prof. T. G. and Mrs. Thompson, Washington and Puget Sound Station (General).
Dr. M. A. Thynne, Plymouth (Nematodes).
A. Walton, Cambridge (Fertilisation studies with Echinus).
Miss M. A. Westbrook, London (Marine Algæ).
Dr. A. G. Wiersma, Utrecht (Peripheral nerve muscle preparations of Crustaceans).

The usual Easter Vacation Course in Marine Zoology was conducted by Mr. D. P. Wilson and Mr. G. A. Steven, and was attended by forty-four students from Oxford, Cambridge, London, Edinburgh, Manchester, Liverpool, Birmingham and Sheffield.

An advanced course in Comparative Physiology and Experimental

Biology was conducted by Mr. A. D. Ritchie and Mr. C. F. A. Pantin during the Summer Vacation and was attended by fourteen students.

During the Easter Vacation, Mr. J. M. Branfoot brought four students from Oundle School and two from Wellington College; Dr. H. Fogg brought two students from Malvern College; Mr. A. S. Gillespie conducted a class of ten students from Dauntsey School; Mr. A. S. Turner brought one student from Rydal School; Mr. H. P. Ramage brought four students from Gresham School, and Mr. Pask brought eight students from St. Paul's School.

At Whitsuntide Mr. Leigh-Sharpe, of the Chelsea Polytechnic, conducted a class of eight students.

General Work at the Plymouth Laboratory.

Herring biology and the winter drift-net fishery at Plymouth have again occupied the major part of Mr. Ford's attention. During the winter of 1929–30, samples from the commercial landings consistently showed a predominance of fish derived from the spawning of the winter 1924–25. That this would be so was predicted two years ago on the evidence of samples collected during the season 1927–28. A successful year-class can be detected at the age of three years, when it enters the Plymouth commercial catches for the first time, and it will attain maximum importance to the fishery at the age of five years. The fishery itself will be most fruitful in yield when a highly successful year-class is present in the form of five-year-old fishes. Certainly the season 1929–30 was a good one; severe gales alone robbed the fishery of excellent results. One unusual event worth recording is that a considerable quantity of herring spawn was taken in the trawl by s.s. *Salpa* on March 3rd, 1930. The estimated number of eggs taken was from $1\frac{1}{2}$ to 2 millions, and they were adhering to shell-gravel to form a sheet, three to four eggs deep. It should also be noted that spawning during the 1929–30 season must have continued over an unusually protracted period, for as late as May 14th the *Salpa* brought to the Laboratory eleven herrings in a spawning condition. The fact that herrings may actually spawn in the vicinity of Plymouth during eight calendar months, from October to May, is a highly important one.

A report has been published on the differential growth of young herrings during the transition from transparent larvæ to fully-scaled and silvery adolescence. The results have significance in their bearing upon the problems raised by the conception of hereditary races of herrings. They suggest that although real biometric differences are observable between random samples of herrings from commercial catches, the biological interpretation of such differences remains open to question,

and seems likely to remain so until experimental work in rearing herrings from parents of known character has yielded fundamentally important information regarding the rôle played by heredity and environment in setting the morphological characters of a new generation.

A paper has also been published by Mr. Ford in which he describes and figures a number of interesting abnormalities in fishes. Several of these serve to demonstrate how fishes may continue to live and thrive in spite of seemingly serious malformations.

The experiments on the growth of plaice in the pond at Pier Cellars, Cawsand Bay, undertaken for the Ministry of Agriculture and Fisheries, were continued by Mr. B. Dawes and Miss D. Thursby-Pelham and, for the last few weeks of the season, completed by Messrs. G. M. Spooner and J. E. Smith. Mr. Dawes has published in the Journal a paper on the work of the first two years, giving data, for second-year fish of various sizes, (*a*) on the daily ration sufficient to keep body-weight constant, and (*b*) on the relation between growth and food taken in excess of maintenance requirements. The work at Cawsand is also compared with similar work carried out on the Exe. The experiments this year were designed with a view to confirming these results and making them as complete as possible.

During the early part of the year Mr. G. A. Steven's researches upon the Bottom Fauna and Fish Food on the "corner" fishing-ground off Plymouth were completed and the results published in the Journal of the Association. Since then, observations have been made on the feeding habits and migrations of the turbot. A gregarious and migratory species, this fish appears in numbers in Plymouth waters at the same time as the herring, upon which it depends almost entirely for food. When the last of the herrings depart, the turbot also disappear, possibly accompanying the herring shoals on their migrations.

Researches have since been commenced by Mr. Steven on the general life-history and biology of rays and skates, elasmobranch fishes about which very little is known in spite of the fact that they are now of great economic importance. In Devon and Cornwall they form the greater part of the landings from about the end of May until October or November. Observations have been made on Plymouth and Newlyn fish markets and several trips have been taken with commercial fishing vessels for the purpose of ascertaining the number of species of Raia which are caught within this area, their relative abundance, size, and general distribution. The relative proportions of the sexes in each of the eleven species landed have been noted. In *Raia clavata* definite evidence of the segregation of the sexes into separate shoals has been obtained. Efforts are also being made to acquire some knowledge of the growth-rate and migrations of these fishes. For this purpose special marks have been devised with which it is hoped to carry out marking experiments during the coming

year. Attempts to discover where the eggs of the Raiidae are deposited have as yet proved unsuccessful but will be continued. Full egg-capsules have never been taken in quantity from the sea.

In co-operation with the Cornish Sea Fisheries Committee, Mr. V. C. Wynne-Edwards, assisted by Mr. G. A. Steven, has commenced an investigation into the food of shags and cormorants. The evidence so far obtained does not support the view that these birds prey to any appreciable extent upon fishes of economic importance, but the data acquired being as yet inadequate it is hoped to continue the observations next year.

Mr. G. M. Spooner has been engaged on an investigation of the phenomenon of schooling in fish, especially from the point of view of the behaviour of the individual fishes of a school. Among the very few species available for laboratory work, the bass (*Morone labrax*) proved suitable. Experiments with mirrors showed that the fish reacted to their own reflections in the same way as to one another, and the use of sight alone will account for all observed schooling behaviour. Investigation of the anatomy of the brain has shown that the optic centres are well developed.

Preliminary experiments on the behaviour of young herrings and other young fish during the first few days of life were carried out and indicated how thoroughly at the mercy of their surroundings the fish are at this stage.

The work on the Mendelian inheritance of eye-colour in *Gammarus* which Mrs. Sexton has carried on for some years with the assistance of Miss A. R. Clark, has made considerable advance during the year, much useful help on the theoretical side having been given by Mr. G. M. Spooner. A paper in which these three workers collaborated appeared in the number of the Journal published in September. When animals brought in from the wild were kept under similar conditions, at temperatures ranging from 20° C. to 28° C., two-thirds of the pairs gave red-eyes in the offspring. One family of these (Mutant Stock V) was worked out in detail for eight or nine generations. The eye-redness was of a complicated kind. All intermediate stages between black and red have occurred. Changes undergone after birth include both darkening and lightening, as well as either permanent or temporary changes to some shade of purple. It is however possible to express all colour variations occurring in the stock in terms of variations in the concentration of the two pigments present in the normal eye, the red and the black. It is a matter of importance that the eye-redness found in the five different stocks in which detailed work has been done is in each case genetically distinct, and any cross-mating between the reds of the different stocks produced black-eyed offspring in the first generation.

Dr. Lebour has spent much time on the revision of the Plymouth Fauna List, which is now being prepared for publication. In March her book *The Planktonic Diatoms of Northern Seas* was published by the Ray Society, and in May a paper, "The Larval Stages of Caridion with a description of a new species, *C. steveni*," appeared in the Proceedings of the Zoological Society of London. Continuing her work on larval decapods, a paper is finished on the larvæ of the Crangonidæ and Hippolytidæ, containing figures and descriptions of most of the British species. All the Plymouth species have now been hatched from the egg. A paper on further observations on the Plymouth Brachyura is also finished, with a description of the little-known zoeae of *Pisa gibbsi* hatched from the egg. Both these papers, with coloured illustrations, are in course of publication by the Zoological Society in their Proceedings. A paper on the larvæ of the Plymouth Galatheidæ was published in the last number of the Association's Journal. The larvæ of all the local species have been identified with the exception of one doubtful one, and most of them have been hatched from the egg.

Preliminary work on the larval mollusca of the plankton has brought forward many interesting problems. The spawning of both the pteropods *Limacina retroversa* and *Clione limacina* has been observed and it can be shown that both species are much more abundant locally than was previously thought, being regular members of the plankton with maxima and breeding seasons in summer. Both have been hatched from the egg and the larvæ of Clione reared until the stage with three ciliated rings after it has lost its shell, the other stages being found in the plankton. The larval period is short and the adults begin to spawn here at a very small size, mostly from 4 to 5 mm.

Attention has been specially given by Dr. Lebour to the larval planktonic gastropods and it is found that *Nassa incrassata* is very important in early summer, appearing in most of the plankton hauls as a handsome veliger with a four-lobed velum. These veligers were reared in a plunger-jar until the adult form could be recognised. Adults collected in late summer laid eggs on Cellaria in a plunger-jar in November. The larvæ from these are hatched with a velum very different from the older planktonic larvæ. It is hoped to rear them in order to follow the change in form. Rissoa spawn and larvæ are receiving special attention, and it has been found that it is almost certainly a newly hatched Rissoa which always forms a large part of the food of the larval herring in the winter.

In working out his observations on the vertical distribution of plankton Mr. F. S. Russell has been giving special attention to the behaviour and biology of Sagitta. He finds that there are two species occurring commonly at Plymouth, *Sagitta elegans* Verrill and *Sagitta setosa* J. Müller; these two species had previously been confused with *Sagitta bipunctata* Quoy

and Gaimard. In May of this year also two specimens of *Sagitta serratodentata* Krohn were taken near the Eddystone. A study of the diurnal vertical movements of *Sagitta elegans* shows that in the daytime the younger individuals appear to withstand a wider range of intensities of light than the older and larger individuals. At dusk the smaller stages have migrated right to the surface, while the largest forms have shown little movement; intermediate types of movement are shown by the various stages lying between the two extremes. At dawn the larger individuals leave the surface first.

A comparison of the movement of these organisms and the changes in light intensity during the twenty-four hours, computed from data obtained on the continuous light recorder on an exactly similar day, shows that these, and probably other plankton animals, leave the surface some time before the intensity has reached that at which they normally live in the daytime. This suggests that the animals become adapted to increasing light intensities, and that in the early morning, after the darkness of the night, they are more sensitive to light than they are later in the day. This would indicate a shifting optimum intensity during the daytime.

Mr. Russell has published in the Association's Journal a record of the average monthly abundance of the pelagic young of most of the species of fishes in the Plymouth region for the last six years, and is continuing routine weekly collections of young fish. It is hoped that by continuous records such as these it may be possible to detect abnormal years and correlate them with hydrographical and other data.

Mr. D. P. Wilson's researches this year have been confined almost entirely to the study of the Mitraria larva of *Owenia fusiformis*. During the early part of the year material obtained by rearing during the summer of 1929 was sectioned, and its study yielded interesting information about the histology of the remarkable cataclysmic metamorphosis which this larva undergoes. It also revealed the main features of the development of various structures during the earlier stages. It was realised, however, that to complete the work in detail further rearings would be necessary, for the development is long and complex and the 1929 material did not cover it at all stages. Accordingly the summer of 1930 was devoted to rearing more larvæ and to further observations on them when alive. This has yielded an unexpected result with regard to the conditions under which metamorphosis normally takes place, for it happened that one of the cultures was especially successful and produced numerous late larvæ in a very vigorous and healthy condition. As only a few of these, on reaching the critical stage, metamorphosed, experiments were carried out in which larvæ were removed to small vessels and supplied with various grades of bottom deposits. These experiments conclusively

proved that the presence of some form of grit on the bottom of the vessels is necessary before metamorphosis will take place. In clean vessels larvæ would live for days without metamorphosing, but would soon settle if fine sand were supplied. In this connection it should be noted that whereas during earlier stages healthy larvæ always swam strongly up to the surface, as soon as they were ready to metamorphose they showed an equally strong tendency to sink to the bottom and could only be kept off it by the action of the plunger-plate.

The main structural changes involved in metamorphosis take half a minute or less to complete, during which period the truly pelagic larva is changed into a typical benthic worm engaged in swallowing the remains of its prototroch and other larval tissues. In a few minutes it has secreted a mucus tube to the outside of which sand grains have adhered.

The continuous records of hyprographic data between Plymouth and Ushant, commenced in 1921 by the Association, have been maintained during the year by Mr. H. W. Harvey and Mr. G. A. Steven, and continue to be published in the Atlantic Report of the International Council. The year 1930 has been remarkable for the occurrence of water in the neighbourhood of the Scillies having a high salinity of 35·7°/$_{oo}$, characteristic of more southern latitudes and possibly of Mediterranean origin.

Mr. Harvey has continued work on the utilisation of ammonium and other salts by marine diatoms, it having been established that ammonium salts are present in the upper layers of the sea off the Norwegian coast and in the Iceland–Greenland area when all, or practically all, the dissolved nitrate has been utilised by phytoplankton. Preliminary experiments, which it is hoped to continue next season, have indicated that the inshore diatom *Nitzschia closterium* can utilise ammonium in place of nitrate and arsenate in place of phosphate, but satisfactory results have not yet been obtained with a typical open-sea diatom.

During the summer breeding season from May to October, by arrangement with the Ministry of Agriculture and Fisheries, Mr. Harvey was engaged in investigating the breeding of oysters in the Ministry's experimental tanks at Conway, during the absence of Mr. H. P. Sherwood in Canada. A heavy fall of spat was obtained in the two largest tanks in which male plants of *Fucus vesiculosus* had been placed with the object of providing food of suitable size for the free-swimming larvæ. Many experiments were also made with larvæ in small vessels, antherozoids or alga cultures being added as food. In two of these experiments larvæ settled to complete metamorphosis where antherozoids had been supplied. Although the season's results only suggest but give no proof that the spat-fall may have been due to the supply of antherozoids as food, a line of attack is opened up for future investigation.

River Tees Survey.

The biological and chemical survey of the estuary of the River Tees, commenced in April 1929 for the Department of Scientific and Industrial Research, has been continued throughout the year. At the end of September Mr. W. B. Alexander, Superintendent of the Laboratory at Middlesbrough, resigned to take up an appointment at Oxford. His services have proved most valuable. The duties of the Superintendent were taken over by Dr. B. A. Southgate, Mr. R. Bassindale of the University of Sheffield being appointed to continue the biological investigations. Mr. H. W. Harvey has visited Middlesbrough from time to time, and remains in contact with the various problems under investigation there by such visits and by correspondence.

A survey has shown that fixed animals, plants, burrowing and crawling animals are plentiful at both ends of the estuary but few in the central part. Here they are subjected to the greatest variations of salinity and also to the effects of pollution by sewage and by toxic industrial effluents. In order to determine whether this scarcity of animals in the central part of the estuary was due to pollution or was the natural result of rapid and considerable changes of salinity with each ebb and flood of the tide, a survey of the fauna and flora of the unpolluted Firth of Tay was made in August. A very similar distribution was found, and it is clear that the scarcity of animals in the central part of the Tees cannot be attributed to pollution, since it is almost equally marked in the Firth of Tay.

On the other hand, a great number of salmon smolt and some brown trout have been found dead or dying in the upper part of the polluted area in the Tees, whiting, sprat, and sand eels in the lower part, and adult salmon in the central part of the polluted area. It is probable that they have died from the combined effects of poisoning and of asphyxiation in the partially deoxygenated water which occupies the polluted area of the estuary. During April, May and June there was a heavy mortality of smolt as they migrated downstream and met these waters ; 2426 were actually collected and examined, almost all from the upper part of the area, only 200 being found below Stockton. It is remarkable that the greatest mortality took place at spring tides ; it remains to be determined whether the migration of smolt takes place only at spring tides in this and other unpolluted estuaries.

The effect of typical coke-oven effluents, diluted with fully aerated and with partially deoxygenated water, upon trout and smolt has been investigated, and the relative toxicity of the more poisonous constituents of these effluents ascertained. The effect of one of these constituents was found to override that of the others.

The dissolved oxygen content of the water in the polluted area, centred upon Newport and swinging up and down the river with the tides, has been the subject of continuous study. The temperature of the water is found to be the main controlling factor, rise in temperature increasing the rate of bacterial breakdown of sewage, of mud and of tar acids discharged from coke ovens. The dissolved oxygen may fall to below 20% of saturation during the summer, the minimum value found being 5% in July 1929.

Side by side with the investigation of conditions obtaining in the estuary, an assessment is being made of the " oxygen demand " by the sewage, industrial effluents and mud on the river bottom.

The distribution of salinity throughout the estuary under varying conditions of fresh-water flow indicates the presence, residual to tidal movements, of a circulatory system in which more saline water tends to move up the estuary along the bottom and less saline water to flow seaward in the upper layers. This is in agreement with current measurements. The total volume of fresh water in the estuary under dry summer conditions has been calculated ; from this value and the rate at which fresh water entered the head of the estuary, the time taken by the fresh water—and hence by substances in solution—to reach the sea under such conditions has been estimated.

Department of General Physiology.

Dr. Atkins and Dr. H. H. Poole have continued their collaboration on photo-electric photometry. By combining a sensitive vacuum cell with a Cambridge " Thread Recorder ", daily records of the vertical illumination have been obtained since December 1929. The photometer has been re-standardized at intervals by two methods. It does not remain quite constant, but is not subject to the rapid and large fluctuations in sensitivity shown by the gas-filled cells examined.

Using the General Electric Company's red sensitive cell with colour filters, measurements have been made of the colour of daylight under various conditions ; the method eliminates the wave-length sensitivity curve of the cell used, being based upon the ratios of blue to green, blue to yellow, etc. A preliminary account of this work was communicated to the Physical and Optical Societies last June. It is hoped that it may be possible to apply it to measurements in the sea.

Miss F. A. Stanbury of the Plymouth Technical College has been engaged in work upon the growth of diatoms under light filters which reduce the intensity non-selectively and selectively. The results varied according to the time of year. In bright summer weather optimum multiplication was shown under screens that reduced the daylight very

considerably. Under such conditions the growth appeared to be much the same whether the screen reduced the illumination non-selectively or selectively, provided the energy transmitted was the same. This is more in keeping with the results of Warburg and Negelein than with those of Klugh. The same species of diatom was found to vary from green to dark brown according to the colour of the light in which it had multiplied.

A considerable amount of advice was given during the year upon the preservation of fishing nets. Durability tests were begun upon material treated by a net-making firm and materials under test here were inspected. Dr. Yonge reported that the plankton nets treated with copper soap remained quite unattacked by cockroaches during the Barrier Reef Expedition. This is an important point as cockroaches are so destructive in the Tropics.

A comparison was made of the data for phosphate and silicate in sea-water from 1923 to 1929 in relation to the seasonal changes in the phyto-plankton. The years show a general similiarity, but there is much evidence that phenomena depending mainly upon illumination may get badly out of step with those depending upon temperature alone. Thus it happened that the year with the coldest water in early spring was also the year with the earliest development of phytoplankton. One might therefore expect this year, 1924, to be an outstanding one as regards favourable or unfavourable conditions for young fish.

There is no evidence that it was specially favourable for herring, but the cold weather resulted in a more prolonged spawning period for fish in general, so that young fish were abundant. As regards herring it was the next spawning period, the winter of 1924–spring of 1925, that was exceptionally good according to the results of Mr. Ford and Mr. Hodgson.

Measurements were made of the hydrogen ion concentration of water in contact with various minerals, especially basalts in various stages of weathering. The reaction of fresh water is governed largely by such contacts. This line of attack may be expected to give information of value in relation to the problem of dolomite formation and changes in the calcium magnesium ratio in coral reefs.

Since his appointment in July Dr. L. N. H. Cooper has been engaged upon the analysis of the minor constituents of sea-water, repeating known methods and trying to follow the seasonal changes in ammonium salts by the method of direct nesslerization which had not been used here before.

Dr. C. M. Yonge has continued his work on the feeding, digestion and metabolism of marine invertebrates. He has been engaged on the further examination and the writing up of work carried out on feeding and digestion in corals during the course of the Great Barrier Reef

Expedition. Considerable progress has been made in this work, which represents a logical development of previous work carried out at the Plymouth Laboratory. Two papers (one to be published in Australia) on the organization of the Expedition have been completed, and also three papers, one on feeding, a second on digestive enzymes, and a third on assimilation and excretion in corals, which are all being published by the British Museum (Natural History). Corals, in common probably with all Cœlenterates, are highly specialized carnivores, feeding only on zooplankton and capable only of digesting protein, fats and the solitary carbohydrate (glycogen) found in animal tissues. Work in course of completion on the interrelations between corals and their contained zooxanthellæ indicates that these are intimately bound up with the specialized nature of the digestive processes of the corals.

In addition Dr. Yonge has begun work on the properties of the chitinous membrane which lines the fore-gut of lobsters and other Decapod Crustacea. Preliminary investigations have shown that this dead membrane possesses properties somewhat analogous, in its selective and to some degree polarised permeability, to those of living membranes. Interesting results are expected from this work.

The Library.

The pressure on the available space for shelves in the Library, to which attention was drawn in the Report of the Council last year, has become much more pronounced, but owing to the generosity of those friends of the Association who have subscribed to the Building Extension Fund, this difficulty will soon be overcome.

The thanks of the Association are again due to numerous Foreign Government Departments, and to Universities and other Institutions at home and abroad for copies of books and current numbers of periodicals presented to the Library, or received in exchange for the Journal. Thanks are also due to those authors who have sent reprints of their papers, which are much appreciated.

Published Memoirs.

The following papers, the outcome of work done at the Laboratory, have been published elsewhere than in the Journal of the Association :

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Hobson, A. D. *Regeneration of the Spines in Sea-Urchins.* "Nature," Vol. CXXV, 1930, p. 168.

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Meyer, A. *Über Cölombewimperung und cölomatische Kreislaufsysteme bei Wirbellosen. II.* Teil. *(Sipunculoidea, Polychæta Errantia.) Ein Beitrag zur Histophysiologie und Phylogenese des Cölomsystems.* Zeits. f. wiss. Zool., Bd. CXXXV, 1929, pp. 495–538.

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Finance.

The Council again wish to express their gratitude to the Development Commissioners for their continued support of the work of the Association. They have to thank also for generous grants the Fishmongers' Company (£600), the British Association (£50), the Royal Society (£50), the Physiological Society (£30), the Ray Lankester Trustees (£20), the Universities of Cambridge (£105), Oxford (£52 10s.), London (£52 10s.), Bristol (£25), Birmingham (£31 10s.), Leeds (£21), Manchester (£21) and Sheffield (£10).

Vice-Presidents, Officers and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1931–32 :—

President.

Lieut.-Col. The Right Hon. WALTER E. GUINNESS, D.S.O., M.P.

Vice-Presidents.

The Duke of BEDFORD, K.G.
The Earl of STRADBROKE, K.C.M.G., C.B., C.V.O.
The Earl of IVEAGH, C.B., C.M.G.
Viscount ASTOR.
Lord ST. LEVAN, C.B., C.V.O.
The Right Hon. Sir AUSTEN CHAMBERLAIN, K.G., M.P.
Lord NOEL BUXTON, M.P.
Sir W. B. HARDY, F.R.S.
GEORGE EVANS, Esq.
Sir NICHOLAS WATERHOUSE, K.B.E.
G. A. BOULENGER, Esq., F.R.S.
J. O. BORLEY, Esq., O.B.E.

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C. TATE REGAN, Esq., F.R.S.
A. D. RITCHIE, Esq.
G. C. ROBSON, Esq.
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J. M. TABOR, Esq.
Prof. D. M. S. WATSON, F.R.S.

Chairman of Council.

Prof. E. W. MACBRIDE, D.Sc., F.R.S.

Hon. Treasurer.

NIGEL O. WALKER, Esq., 38, Regent Street, Cambridge.

Hon. Secretary.

E. J. ALLEN, Esq., D.Sc., F.R.S.,
The Laboratory, Citadel Hill, Plymouth.

The following Governors are also members of the Council :—

G. P. BIDDER, Esq., Sc.D.
E. T. BROWNE, Esq.
GEORGE EVANS, Esq.
H. G. MAURICE, Esq., C.B. (Ministry of Agriculture and Fisheries).
Sir A. GARRETT ANDERSON, K.B.E. (Prime Warden of the Fishmongers' Company).
NIGEL O. WALKER, Esq. (Fishmongers' Company).
LOTHIAN D. NICHOLSON, Esq. (Fishmongers' Company).
Prof. G. C. BOURNE, D.Sc., F.R.S. (Oxford University).
J. GRAY, Esq., F.R.S. (Cambridge University).
Sir P. CHALMERS MITCHELL, Kt., C.B.E., D.Sc., F.R.S. (British Association).
Prof. E. W. MACBRIDE, D.Sc., F.R.S. (Zoological Society).
Sir SIDNEY HARMER, K.B.E., F.R.S. (Royal Society).

Dr. *Statement of Receipts and Payments for the*

GENERAL

	£ s. d.	£ s. d.
To Balance from 31st March, 1930 :—		
Cash in hand	22 19 7	
Cash at Lloyds Bank £176 6 10		
Less Coutts & Co.—Overdraft 68 9 3	107 17 7	130 17 2
,, Grants :—		
Ministry of Agriculture and Fisheries Grant from Development Fund	11,477 11 4	
Fishmongers' Company	600 0 0	
British Association	50 0 0	
Royal Society	50 0 0	
Physiological Society (two years)	60 0 0	12,237 11 4
,, Subscriptions		268 15 0
,, Donations		6 2 0
,, Sale of Specimens		1,286 14 4
,, ,, Fish (*less* Expenses)		121 16 6
,, ,, Nets, Gear, and Hydrographical Apparatus		412 19 10
,, Table Rent (including Cambridge University, £105 ; Oxford University, £52 10s. ; London University, £52 10s. ; Bristol University, £25 ; Birmingham University, £31 10s. ; Leeds University, £21 ; Manchester University, £21 ; Sheffield University, £10 ; Trustees of Ray Lankester Fund, £20)		502 17 3
,, Tank Room Receipts		477 3 9
,, Interest on Investments :—		
4% War Stock	9 10 2	
4% New Zealand Stock	12 14 8	
3% Local Loans	2 0 11	
Interest on Deposits	4 16 5	29 2 2
,, Sale of £237 17s. 11d. 4% War Stock		238 19 7
,, Sale of Dr. M. V. Lebour's Book		5 5 8
,, Grant from Zoological Society towards Publication of Marine Fauna of Plymouth		5 0 0

The Association's Bankers held on its behalf :—
£410 14s. 8d. New Zealand 4%, 1943-63.
£352 2s. 3d. Local Loans 3% Stock.

£15,723 4 7

BUILDING EXTEN

	£ s. d.
To Sundry Donations	2,690 6 11
	£2,690 6 11

Further Liabilities on the Building Fund to the

SUPERANNUATION

	£ s. d.
To Grant from H.M. Treasury	2,687 6 0
	£2,687 6 0

PLAICE EXPERIMENTS

	£ s. d.
To Grant from Ministry of Agriculture and Fisheries	132 4 3
,, General Fund	13 0 4
	£145 4 7

DEPRECIA

	£ s. d.
To General Fund	250 0 0
	£250 0 0

Year 1st April, 1930, to 31st March, 1931. Cr.

FUND.

	£ s. d.	£ s. d.
By Salaries :—		
Director	1,200 0 0	
Physiologist	910 0 0	
Naturalists	3,526 16 9	
Hydrographer	600 0 0	6,236 16 9
,, Laboratory Wages (including National Insurances)		1,970 4 0
,, Annual Upkeep of Library		596 13 0
,, Scientific Publications :—		
Journal, Vols. XVI, No. 3 and XVII, No. 1	812 8 5	
Less Sales	204 15 6	607 12 11
,, Annual Upkeep of Laboratories and Tank Rooms :—		
Buildings and Machinery	392 1 9	
Electricity, Gas, Coal, and Water	338 2 2	
Chemicals and Apparatus	606 15 7	
Rates, Taxes, and Insurance	112 12 6	
Travelling £141 8 11		
,, "Challenger" Society 31 18 11	173 7 10	
Stationery, Postages, Telephone, Carriage, and Sundries	440 6 10	
Purchase of Specimens	122 4 10	2,185 11 6
,, Annual Maintenance and Hire of Boats :—		
Wages (including Diet Allowance, National Health Insurance, and Casual Labour)	1,624 1 1	
Coal, Water, Oil, etc.	216 19 8	
Maintenance and Repairs, with Nets, Gear, and Apparatus	773 13 6	
Purchase of Material for Nets for sale (excluding labour)	198 9 2	
Boat Hire and Collecting Expenses	34 5 9	
Insurance	310 9 2	3,157 18 4
,, Interest on Bank Loan		7 15 0
,, Purchase of £352 2s. 3d. Local Loans 3% Stock		238 19 7
,, Transfer to Plaice Experiments Account		13 0 4
,, Depreciation Fund		250 0 0
,, Balance, 31st March, 1931 :—		
Cash in hand	24 6 6	
Cash at Lloyds Bank	194 11 3	
Cash at Coutts	239 15 5	458 13 2
		£15,723 4 7

SION FUND.

	£ s. d.
By Payment to Builders (Messrs. A. N. Coles & Co. Ltd.)	500 0 0
,, Cash at Coutts on Deposit Account	2,100 0 0
,, Cash at Coutts on Current Account	90 6 11
	£2,690 6 11

amount of £1,953 17s. have been incurred.

FUND.

	£ s. d.
By Cash at Coutts on Deposit	2,687 6 0
	£2,687 6 0

AT CAWSAND.

	£ s. d.
By Salary of Ben Dawes, Esq.	117 10 0
,, ,, ,, J. E. Smith, Esq.	11 2 3
,, Incidental Expenses	16 12 4
	£145 4 7

TION FUND.

	£ s. d.
By Cash at Coutts on Deposit Account	250 0 0
	£250 0 0

Examined and found correct,

3 *Frederick's Place,*
Old Jewry, London, E.C. 2.
27th April, 1931.

(Signed) N. E. Waterhouse, *Auditor.*
L. D. Nicholson } *Members of*
Nigel O. Walker } *Council.*

List of Annual Subscriptions

Paid during the Year, 1st April, 1930, to 31st March, 1931.

	£	s.	d.
Dr. W. M. Aders	1	1	0
Dr. E. J. Allen, F.R.S.	1	1	0
G. L. Alward, Esq.	1	1	0
Dr. Ikusaku Amemiya (1930 and 1931)	2	2	0
Prof. J. H. Ashworth, F.R.S.	1	1	0
The Rt. Hon. Lord Askwith, K.C.B., D.C.L.	1	1	0
Miss D. Atkins	1	1	0
Prof. J. Barcroft, F.R.S. (1928-1930)	3	3	0
W. H. Barrett, Esq. (1931 and 1932)	2	2	0
W. J. Bazeley, Esq. (1929 and 1930)	2	2	0
G. R. de Beer, Esq.	1	1	0
J. Belehradek, Esq., M.D.	1	1	0
N. J. Berrill, Esq.	1	1	0
Mrs. M. G. Bidder	1	1	0
Birkbeck College	1	1	0
Dr. H. Blaschko (1931 and 1932)	2	2	0
H. H. Bloomer, Esq. (1929 and 1930)	2	2	0
Mrs. H. Moss Blundell	1	1	0
Dr. J. Borowik	1	1	0
Dr. L. A. Borradaile	1	1	0
Prof. Gilbert C. Bourne, F.R.S. (1928 and 1929)	2	2	0
Dr. B. G. Bogorov	1	1	0
Col. Sir Henry Bowles, Bart. (1930 and 1931)	2	2	0
Dr. A. Bowman	1	1	0
Prof. A. E. Boycott, F.R.S.	1	1	0
Sir J. Rose Bradford, Bart., K.C.M.G., M.D., F.R.S.	1	1	0
J. M. Branfoot, Esq.	1	1	0
Brighton Public Library	1	1	0
L. R. Brightwell, Esq. (1929-1931)	3	3	0
H. H. Brindley, Esq.	1	1	0
R. Brown, Esq.	1	1	0
Miss E. M. Brown	1	1	0
H. O. Bull, Esq.	1	1	0
R. H. Burne, Esq., F.R.S.	1	1	0
Carried forward	47	5	0

	£	s.	d.
Brought forward	47	5	0
M. Burton, Esq.	1	1	0
R. R. Butler, Esq.	1	1	0
L. W. Byrne, Esq.	1	1	0
Dr. W. T. Calman, F.R.S.	1	1	0
Prof. H. Graham Cannon SC.D. (1930 and 1931)	2	2	0
J. N. Carruthers, Esq.	1	1	0
Paymaster Capt. Charles, R.N. (Retd.)	1	1	0
G. S. R. Kitson Clark (1925-1930)	6	6	0
Dr. James Clark	1	1	0
Coastguard and Fisheries Service, Alexandria	1	1	0
J. S. Colman, Esq.	1	1	0
J. F. Coonan, Esq.	1	1	0
J. Omer Cooper, Esq.	1	1	0
L. R. Crawshay, Esq.	1	1	0
H. H. Dale, Esq., C.B.E., M.D., F.R.S. (1927-1930)	4	4	0
Prof. Otto. V. Darbishire	1	1	0
Dr. H. H. Darby (1930 and 1931)	2	2	0
F. M. Davis, Esq.	1	1	0
Director of Agriculture and Fisheries, Travancore (1928–1930)	3	3	0
F. A. Dixey, Esq., F.R.S.	1	1	0
C. C. Dobell, Esq., F.R.S.	1	1	0
F. Martin Duncan, Esq. (1930 and 1931)	2	2	0
The late Prof. J. S. Dunkerley, D.SC., PH.D.	1	1	0
P. Eggleton, Esq.	1	1	0
C. E. D. Enoch, Esq.	1	1	0
Prof. C. Lovatt Evans, F.R.S.	1	1	0
Col. T. Fetherstonhaugh, D.S.O.	1	1	0
Dr. R. A. Fisher, F.R.S.	1	1	0
Fisheries Survey Committee, Capetown	1	1	0
E. Ford, Esq.	1	1	0
Dr. G. Herbert Fowler (1928–1930)	3	3	0
Prof. H. M. Fox	1	1	0
Miss E. A. Fraser, D.SC.	1	1	0
Prof. F. E. Fritsch	1	1	0
T Fujita, Esq.	1	1	0
R. D'O Good, Esq.	1	1	0
Prof. E. S. Goodrich, F.R.S.	1	1	0
A. P. Graham, Esq.	1	1	0
Michael Graham, Esq.	1	1	0
Carried forward	103	19	0

	£	s.	d.
Brought forward	103	19	0
R. Grant, Esq.	1	1	0
Dr. A. H. M. Gray (1930 and 1931)	2	2	0
Dr. R. W. Gray	1	1	0
Dr. H. P. Hacker	1	1	0
Wilfred Hall, Esq. (1930 and 1931)	2	2	0
Prof. A. C. Hardy	1	1	0
Prof. C. R. Harington, F.R.S.	1	1	0
T. J. Hart, Esq.	1	1	0
H. W. Harvey, Esq.	1	1	0
J. C. Hemmeter, Esq., M.D.	1	1	0
C. C. Hentschel, Esq.	1	1	0
C. F. Hickling, Esq.	1	1	0
Prof. A. V. Hill, F.R.S.	1	1	0
Prof. J. P. Hill, F.R.S.	1	1	0
W. T. Hillier, Esq., M.R.C.S.	1	1	0
Prof. K. Hirasaka (1929 and 1930)	2	2	0
A. D. Hobson, Esq. (1928–1930)	3	3	0
Prof. L. T. Hogben, D.SC. (1928–1930)	3	3	0
Dr. E. G. Holmes	1	1	0
F. R. Horne, Esq.	1	1	0
Capt. G. C. L. Howell	1	1	0
P. Hoyte, Esq. (1929 and 1930)	2	2	0
Hull Univeristy College (1930 and 1931)	2	2	0
O. D. Hunt, Esq.	1	1	0
Prof. J. S. Huxley (1928–1930)	3	3	0
Lt. H. L. Jenkins, R.N.	1	1	0
J. J. Judge, Esq.	1	1	0
Dr. Stanley Kemp, F.R.S.	1	1	0
Mrs. A. Redman King	1	1	0
J. A. Kitching, Esq.	1	1	0
Dr. E. M. Kreps	1	1	0
Dr. G. Lapage	1	1	0
Prof. R. D. Laurie	1	1	0
A. G. Lowndes, Esq.	1	1	0
J. R. Lumby, Esq.	1	1	0
Adrian Lumley, Esq. (1931 and 1932)	2	2	0
H. Macarthur, Esq.	1	1	0
Prof. E. W. MacBride, F.R.S.	1	0	0
G. I. Mann, Esq.	1	1	0
Carried forward	157	9	0

	£	s.	d.
Brought forward	157	9	0
B. J. Marples, Esq.	1	1	0
D. J. Matthews, Esq.	1	1	0
H. G. Maurice, Esq., C.B. (1923–1930)	8	8	0
Capt. W. N. McClean	1	1	0
A. P. Menon, Esq.	1	1	0
Milford Haven Trawler Owners and Fish Salesmen's Association, Ltd.	1	1	0
W. S. Millard, Esq.	1	1	0
Sir P. Chalmers Mitchell, Kt., C.B.E., F.R.S.	1	1	0
F. W. Moorhouse, Esq.	1	1	0
C. C. Morley, Esq.	1	1	0
Dr. J. Mukerjii	1	1	0
National Museum of Wales	1	1	0
R. G. Neill, Esq. (1929 and 1930)	2	2	0
A. G. Nicholls, Esq.	1	1	0
J. R. Norman, Esq.	1	1	0
Charles Oldham, Esq.	1	1	0
G. W. Olive, Esq.	1	1	0
Prof. J. H. Orton, D.SC.	1	1	0
R. Palmer, Esq.	1	1	0
The Hon. John H. Parker	1	1	0
C. W. Parsons, Esq.	1	1	0
Pawlyn Bros.	1	1	0
T. A. Pawlyn, Esq.	1	1	0
F. T. K. Pentelow, Esq.	1	1	0
Pease Laboratories	1	1	0
Plymouth Corporation Museum Committee (1928-1930)	3	3	0
Plymouth Education Authority (1929 and 1930)	2	2	0
Plymouth Public Library	1	1	0
Plymouth Proprietary Library	1	1	0
Port of Plymouth Incorporated Chamber of Commerce	1	1	0
Portsmouth Municipal College	1	1	0
W. Proctor, Esq. (1929 and 1930)	2	2	0
D. M. Reid, Esq.	1	1	0
E. A. Robins, Esq.	1	1	0
C. H. Rudge, Esq. (1930 and 1931)	2	2	0
Dr. E. S. Russell	1	1	0
F. S. Russell, Esq., D.S.C., D.F.C.	1	1	0
Capt. the Hon. Lionel St. Aubyn, M.V.O. (1930 and 1931)	2	2	0
The Rt. Hon. Lord St. Levan, C.B., C.V.O.	1	1	0
Carried forward	213	2	0

	£	s.	d.
Brought forward	213	2	0
J. T. Saunders, Esq.	1	1	0
R. E. Savage, Esq. (1930 and 1931)	2	2	0
F. W. Schiller, Esq. (1928–1929)	2	2	0
Dr. Edgar Schuster	1	1	0
W. L. Sclater, Esq.	1	1	0
B. Sen, Esq.	1	1	0
Lt.-Col. R. B. Seymour Sewell, I.M.S. (1930 and 1931)	2	2	0
Miss Lilian Sheldon	1	1	0
N. Smedley, Esq. (1930 and 1931)	2	2	0
B. Webster Smith, Esq.	1	1	0
F. G. W. Smith, Esq.	1	1	0
G. M. Spooner, Esq.	1	1	0
States Committee for Fisheries, Guernsey	1	1	0
A. C. Stephen, Esq.	1	1	0
Dr. T. A. Stephenson (1930 and 1931)	2	2	0
Mrs. N. S. Steven	1	1	0
The Rt. Hon. the Earl of Stradbroke, K.C.M.G., C.V.O., C.B.	1	1	0
The late H. H. Sturch, Esq.	1	1	0
Eric. J. Tabor, Esq.	1	1	0
Harold E. Tabor, Esq.	1	1	0
J. M. Tabor, Esq.	1	1	0
Prof. W M. Tattersall, D.SC.	1	1	0
Sir Charles Howell Thomas, K.C.B. (1930 and 1931)	2	2	0
Mrs. M. A. Thynne, M.R.C.S.	1	1	0
Torquay Natural History Society	1	1	0
R. C. Vernon, Esq.	1	1	0
Nigel O. Walker, Esq. (1928–1930)	3	3	0
A. Walton, Esq.	1	1	0
B. H. Walton, Esq. (1928-1930)	3	3	0
Alan H. Ware, Esq., PH.C.	1	1	0
Sir. Nicholas E. Waterhouse, K.B.E. (1928-1930)	3	3	0
Prof. D. M. S. Watson, F.R.S.	1	1	0
E. C. Weberman, Esq.	1	1	0
W. H. Webster, Esq.	1	1	0
Mrs. F. J. Weldon	1	1	0
Miss M. A. Westbrook	1	1	0
Dr. K. B. Williamson	1	1	0
D. P. Wilson, Esq.	1	1	0
R. S. Wimpenny, Esq.	1	1	0
Ronald Winckworth, Esq.	1	1	0
V. C. Wynne-Edwards	1	1	0
Total	£268	15	0

List of Donations to the General Fund

For the Year, 1st April, 1930, to 31st March, 1931.

	£	s.	d.
Bournemouth Corporation	1	1	0
Dr. R. T. Jackson	4	0	0
G. Tregelles, Esq.	1	1	0
Total	£6	2	0

List of Donations towards the Building Extension Fund

Paid during the Year 1st April, 1930, to 31st March, 1931.

	£	s.	d.
Lieut.-Col. The Rt. Hon. Walter E. Guinness, D.S.O., M.P.	1,000	0	0
Dr. G. P. Bidder	500	0	0
E. T. Browne, Esq.	500	0	0
Anonymous	100	0	0
The Rt. Hon. the Earl of Iveagh, C.B., C.M.G.	100	0	0
Mrs. E. T. Browne	50	0	0
A. D. Ritchie, Esq.	33	8	0
The Worshipful Company of Clothworkers	25	0	0
L. K. Elmhirst, Esq.	25	0	0
"Discovery" Committee, Colonial Office	20	0	0
The Rt. Hon. Lord St. Levan, C.B., C.V.O.	20	0	0
B. H. Walton, Esq.	10	10	0
Dr. W. R. G. Atkins, F.R.S.	10	0	0
Prof. G. C. Bourne, F.R.S.	10	0	0
K. Furusawa	10	0	0
Mr. and Mrs. W. Russell	10	0	0
Dr. C. Bolton, F.R.S.	5	5	0
R. H. Burne, Esq., F.R.S.	5	5	0
L. W. Byrne, Esq.	5	5	0
The Great Grimsby Coal, Salt and Tanning Co.	5	5	0
Sir Herbert F. Thompson, Bart.	5	5	0
H. G. Wells, Esq.	5	5	0
Dr. E. J. Allen, F.R.S.	5	0	0
Miss A. Bidder	5	0	0
Col. Sir Henry Bowles, Bart.	5	0	0
L. R. Crawshay, Esq.	5	0	0
Carried forward	2,475	8	0

	£	s.	d.
Brought forward	2,475	8	0
George Evans, Esq.	5	0	0
James Gray, Esq., F.R.S.	5	0	0
Prof. E. S. Goodrich, F.R.S.	5	0	0
Sir Sidney F. Harmer, K.B.E., F.R.S.	5	0	0
Sir John D. Marsden, Bart.	5	0	0
Prof. E. W. MacBride, F.R.S.	5	0	0
Prof. W. C. McIntosh, F.R.S.	5	0	0
Sir P. Chalmers Mitchell, Kt., C.B.E., F.R.S.	5	0	0
Dr. Yo Okada	5	0	0
Charles Oldham, Esq.	5	0	0
G. W. Otter, Esq.	5	0	0
C. F. A. Pantin, Esq.	5	0	0
Prof. Edward B. Poulton, F.R.S.	5	0	0
Messrs. John Staniar & Co.	5	0	0
Prof. W. M. Tattersall, D.SC.	5	0	0
Prof. D. M. S. Watson, F.R.S.	5	0	0
Mrs. F. J. Weldon	5	0	0
L. E. Bayliss, Esq.	3	3	0
Dr. E. A. Fraser	3	3	0
Prof. D. L. Mackinnon, D.SC.	3	3	0
Miss E. A. T. Nicol, PH.D.	3	3	0
Prof. A. V. Hill, F.R.S.	3	0	0
Jiro Murakami, Esq.	3	0	0
D. P. Wilson, Esq.	3	0	0
Prof. J. H. Ashworth, F.R.S.	2	2	0
Dr. J. Borowik	2	2	0
Prof. and Mrs. H. Graham Cannon	2	2	0
Mr. and Mrs. R. S. Clark	2	2	0
Messrs. Coates & Co. (Plymouth), Ltd.	2	2	0
Dr. H. H. Darby	2	2	0
The late Prof. J. S. Dunkerly, D.SC., PH.D.	2	2	0
Messrs. Elliott & Garrood, Ltd.	2	2	0
Mr. & Mrs. E. Ford	2	2	0
G. Herbert Fowler, Esq., PH.D.	2	2	0
Messrs. R. Friedlander & Sohn	2	2	0
Gourock Ropework Company	2	2	0
A. D. Hobson, Esq.	2	2	0
Dr. Stanley Kemp, F.R.S.	2	2	0
Miss S. M. Manton, M.A., PH.D.	2	2	0
Carried forward	2,613	10	0

	£	s.	d.
Brought forward	2,613	10	0
Prof. J. H. Orton, D.SC.	2	2	0
G. P. Wells, Esq.	2	2	0
Prof. J. Barcroft, F.R.S.	2	0	0
Miss. E. W. Emmart	2	0	0
Robert Gurney, Esq.	2	0	0
Prof. A. V. Hill, F.R.S. (second donation)	2	0	0
J. Arthur Hutton, Esq.	2	0	0
F. S. Russell, Esq., D.S.C., D.F.C.	2	0	0
G. M. Spooner, Esq.	2	0	0
Prof. A. Willey, D.SC., F.R.S.	2	0	0
Messrs. E. S. Rendle & Son	1	10	0
A. C. Stephen, Esq.	1	1	3
Messrs. T. & R. Annan & Son	1	1	0
Dr. C. Amirthalingam, Esq.	1	1	0
G. T. Atkinson, Esq.	1	1	0
The Rt. Hon. Lord Askwith, K.C.B., D.C.L.	1	1	0
Dr. L. A. Borradaile	1	1	0
Dr. A. Bowman	1	1	0
R. R. Butler, Esq.	1	1	0
Lord Clwyd	1	1	0
J. S. Colman, Esq.	1	1	0
Capt. G. C. C. Damant, R.N., G.B.E.	1	1	0
Dauntsey School	1	1	0
Messrs. Foster Mason & Harvey	1	1	0
Prof. H. Munro Fox	1	1	0
Prof. R. R. Gates	1	1	0
Dr. R. W. Gray	1	1	0
Prof. A. C. Hardy	1	1	0
Mrs. A. C. Hardy	1	1	0
C. C. Hentschel, Esq.	1	1	0
Prof. E. Hindle	1	1	0
W. T. Hillier, Esq., M.R.C.S.	1	1	0
H. E. Hurrell, Esq.	1	1	0
Prof. H. G. Jackson	1	1	0
J. A. Kitching, Esq.	1	1	0
Messrs. H. K. Lewis & Co., Ltd.	1	1	0
Lowestoft Fishing Vessel Owners Association, Ltd.	1	1	0
R. Macdonald, Esq.	1	1	0
G. I. Mann, Esq.	1	1	0
Carried forward	2,664	12	3

	£	s.	d.
Brought forward	2,664	12	3
H. G. Maurice, Esq., C.B.	1	1	0
R. Margeria, Esq.	1	1	0
W. S. Millard, Esq.	1	1	0
A. G. Nicholls, Esq.	1	1	0
C. W. Parson, Esq.	1	1	0
Dr. C. Tate Regan, F.R.S.	1	1	0
H. C. Regnart, Esq.	1	1	0
D. M. Reid, Esq.	1	1	0
Dr. H. N. Roy	1	1	0
G. A. Steven, Esq.	1	1	0
Miss E. M. Stevens	1	1	0
Dr. and Mrs. K. B. Williamson	1	1	0
Miss M. A. Westbrook	1	1	0
V. C. Wynne-Edwards, Esq.	1	1	0
Prof. A. E. Boycott, F.R.S.	1	0	0
Miss E. M. Brown	1	0	0
Dr. J. N. Carruthers	1	0	0
Dr. P. Eggleton	1	0	0
Miss M. V. Lebour, D.SC.	1	0	0
Miss Yvonne Lebour	1	0	0
R. Palmer, Esq.	1	0	0
R. S. Wimpenny, Esq.	1	0	0
Messrs. Harrington Bros.		10	6
Dr. J. Wyman		10	2
A. P. Graham, Esq.		10	0
T. J. Hart, Esq.		10	0
Miss Ross		10	0
R. C. Vernon, Esq.		10	0
Total	£2,690	6	11

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A Preliminary Comparison of the Neon Lamp and Potentiometer Methods of Submarine Photo-Electric Photometry.

By

W. R. G. Atkins, Sc.D., F.R.S.,

Head of the Department of General Physiology at the Plymouth Laboratory.

And

H. H. Poole, Sc.D.

Registrar of the Royal Dublin Society.

With 2 Figures in the Text.

Our previous experience of submarine photometry (1925, 1926, 1928, 1929) indicated the desirability of obtaining additional information in certain directions and of speeding up the observations without sacrifice of accuracy ; our aim was indeed to increase the accuracy, at the greater depths especially and in sub-surface observations. Further data upon the horizontal illumination, viz. that received by a vertical surface, were also wanted.

The above *desiderata* were sought as follows :

(*a*) To increase accuracy a more sensitive vacuum cell was obtained—a sodium cell of the Burt type, with " end-on " illumination. This had a sensitivity of 5·30 m.c. per 10^{-9} amp. as against 31·6 m.c. for the potassium vacuum cell previously used, photometer L. It was hoped that it would have been possible to use this down to bottom at Station E1, thus avoiding having recourse to a gas-filled cell ; the latter has to be calibrated before and after use and the voltage change-over ratios have also to be determined, so that both on the score of accuracy and speed of operation a vacuum cell is to be preferred when possible. For several reasons, however, the Burt cell could not be used for the deeper water ; firstly, owing to its smaller aperture the " end-on " is not as sensitive as the side window type, secondly, though mounted so that the aperture in the cell was close to a larger window in the new type of photometer case (described later), yet the mounting undoubtedly reduced the sensitivity somewhat, and thirdly, the plate glass, 12·5 mm. thick, reduces the middle of the solar ultra-violet somewhat, yet it is just here—at 360 mμ—that the sodium cell has its greatest sensitivity. In any case the diffusing opal glass must reduce the intensity in this region. Hence

NATIONAL ACADEMY OF SCIENCES,
DEC 5 1931
UNITED STATES OF AMERICA

the sensitivity of this photometer, as given above, proved to be considerably less than was expected. Furthermore, it is possible that the absorption coefficient for the light to which the sodium cell responds is greater than that for the potassium cell, the latter being of greater wave-length. This, however, remains to be tested by the measurements. A greater accuracy throughout, especially in the deeper water, was attained, however, by using photometer J, already described (1929), but maintained in its condition of maximum sensitivity by a method communicated to us by the staff of the Research Laboratories of the General Electric Co. The gas-filled potassium cell, mounted as photometer J, has its sensitivity increased by the passage of a relatively large current caused by exposure to bright light. The subsequent gradual loss of sensitivity when subjected to low illumination, implies that in deep water the cell is not as sensitive as one would expect from the voltage change-over ratio, determined at a lesser depth. For all measurements to be comparable, the sensitivity should be the same. This we formerly sought to maintain by avoiding the passage of a really large current at any time by reducing the applied voltage for bright light, the safe limit being determined by trial. A correction was also introduced, assuming the change of sensitivity to be a linear function of the time. The method, now adopted, is the reverse. The cell is momentarily connected with a voltage just sufficient to produce a glow discharge in absence of light. This leaves it in a highly sensitive state ; immediately after the glow the measurement is made at the voltage appropriate to the illumination. This is the best one can do with a gas-filled cell. A special series of tests was carried out to ascertain the accuracy thus obtainable. These are described elsewhere. (Atkins, 1931.) If the glow were allowed to continue the cell would be ruined. The method gives results which seem to be more constant than those obtained formerly, but not as constant as with a vacuum cell. With light of low illumination, however, the far greater sensitivity of the gas-filled cell outweighs this defect and the readings become more accurate, being much larger.

To sum up, for accuracy and speed of work down to moderate depths the Burt cell (photometer M) or the potassium vacuum cell (photometer L) may be used, but for the greater depths the potassium gas-filled cell (photometer J) must be used. What these depths actually are depends upon the clearness of the water at the time.

(*b*) Observations immediately above and below the water surface are obviously desirable because owing to the shadow of the ship the light just above the water is always less than that as measured on the deck-house roof. Measurements in the latter position serve, however, to detect any change in general illumination. The shadow error is not a constant. The stern of the ship is always kept pointing towards the sun, so that with a

bright sun—say $\beta=3$—the shadow is relatively unimportant, since only a percentage of the diffuse light is cut off. With overcast sky, $\beta=1$, this loss becomes the same percentage of the total light.

As the photometer is lowered through the water the shadow error increases at first and then decreases. It is obvious, however, that it is soon rendered insignificant by the absorption of the water, so though it may affect the percentage values of the illumination at the lesser depths it will have but little effect on the absorption coefficients calculated from them.

The difference between the measurements just above and just below the water surface denotes the loss due to irregular surface reflections, the submarine photometer—even when in air—having a few millimetres of water over its opal disc. Formerly we allowed 15 per cent to cover these shadow and reflection losses and took it that the sub-surface light was 85 per cent of that on the deck-house roof, on an average.

Under calm weather conditions with diffuse light and opal disc over photometer it is quite possible to obtain tolerably good measurements just above and just below the water, using the potentiometer method of measuring the photo-electric current. (See Series 48.) As a rule, however, the conditions render such measurements impossible, because the photometer swings too much, the wave motion is too great at the surface, and the sunlight just below the surface is naturally very variable.

For such measurements the neon lamp method described by J. H. J. Poole (1928) has many advantages, since it gives a measure of an integrated current over any convenient period of time. The measurements made with it in Lower Lough Bray have already been described (J. H. J. and H. H. Poole, 1930). Photometer M was used for this work. The neon lamp outfit taken to sea was the same one that had been at Lough Bray. Since its serviceableness on ships and small boats is great, while its cost and weight are low, an outline of the principle upon which it works is given further on. The fact that the method integrates the current makes it immaterial whether the latter varies irregularly, provided that over the period of the measurements the mean current remains constant. In other words the general illumination must not change—or if it does a series of integration measurements must be performed and an average taken, just as in the potentiometer method. By the help of the neon lamp method measurements were obtained just above and just below the water surface and at depths down to 5 m.; concordant measurements are not easily obtained at depths less than 5 m., using the potentiometer method.

(*c*) Series relating to the horizontal light intensity and the intensity of light travelling upwards have already been given (1928, 1929). These refer to light received on a photometer window placed vertically, or

inverted. Williams (1929) has since made similar measurements with a projecting tube to ensure that only light at 90° to the vertical should enter. Further measurements appeared desirable and as a new case to contain the Burt cell had to be made, it was so designed as to be better adapted for being slung with window vertical. This could most

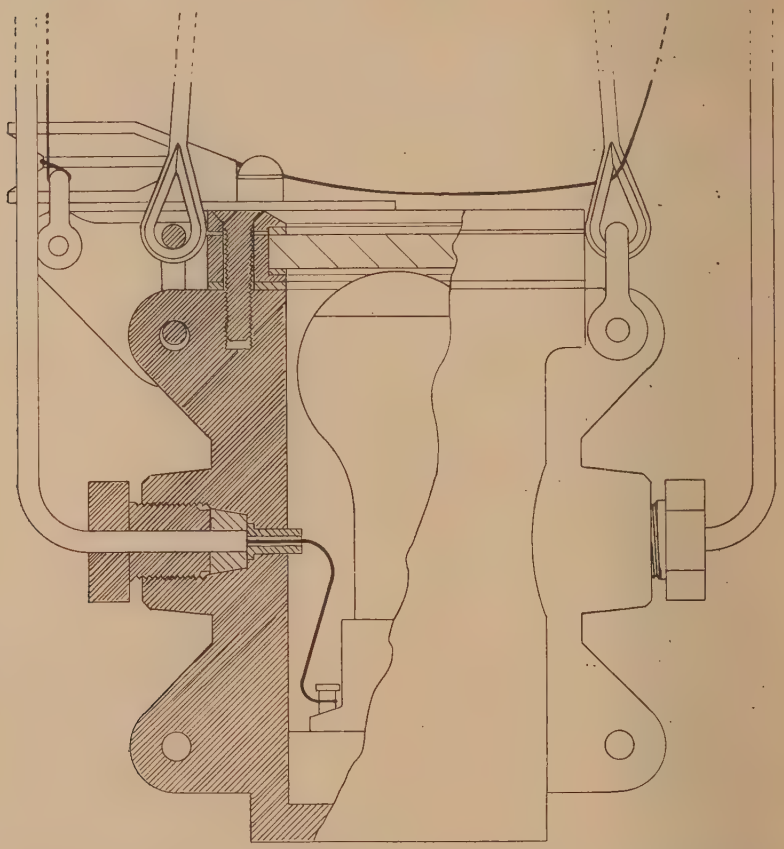

FIG. 1.—Above is shown, in part section, the new type of submarine photometer case with photo-electric cell, the height of the latter, with standard U.S.A. radio valve base, being 16 cm. Immediately over the window of the cell comes the plate glass window, 12·5 mm. thick, which is fixed as described in the lid, one screw of which is shown. The cable leads enter at the sides. Note the lower bolt-holes for lateral or inverted suspension. The photometer is shown with window cover swung clear by applying tension to the twine (full black line) on left-top corner. The twine works in the groove of the wooden projecting piece, which is a cut-away sector of a disc. It is necessary to have the twine passing through a shackle mounted on a metal flange to secure adequate leverage. The insertion of the cables has been described for the earlier type. The opal disc, attached by wax above the plate glass window, is not shown.

conveniently be done by having a cylindrical case (see figure, where the case is shown in part section) and using an "end-on" rather than a side exposure cell. This design was slung by two diametrically opposed steel wires, instead of by four—one from each corner of a box. Moreover, additional bolt-holes were placed, as indicated, near the bottom of the case so that by merely changing one suspending wire and passing its bolt through one of the side lower bolt holes one could sling the photometer with window vertical, instead of horizontal. By using both the lower bolt holes the photometer may be slung in an inverted position. In former measurements the alteration in position had to be made by lashing, with consequent delay and some uncertainty as to a precise setting of the photometer. Figure 1 shows the new type of case. It was lighter and rather easier to handle than the old one. Its greater length, however, made it more apt to tilt in the water were the ship to drift rapidly. This must have been without practical importance in the work here described.

MEASUREMENT OF THE PHOTO-ELECTRIC CURRENT.

(*a*) The Potentiometer Method.

This has been used as previously described. Occasionally with large currents the drop in potential was measured across a 40,000 ohm resistance instead of the usual 100,000 ohm.

In spite of the additional precautions described in our later work (1929) surface leakage continued to be a trouble in very damp weather. The surface of the potentiometer was coated with paraffin wax, with some effect. Nevertheless on October 15, 1929, at Station E 1, with an east wind blowing into the deck-house the leakage was so bad that operations were about to be suspended for the day. As a last resort the insulated metal box containing the H.T. batteries and connected to the negative pole, as shown in the figure facing page 301 (1929), was earthed to a nut on the iron tube carrying the steering chain on the port side of the deck. All troubles ceased immediately. A "dark current" was, however, found again on October 21, but this was traced to photometer M, which on opening was found to have leaked around the window. The internal fittings and cell were washed in distilled water and thoroughly dried; the calcium chloride was renewed; finally a good seal was effected by heating the upper portion and the window holder in an air oven and pouring "SIRA" (Scientific Instrument Research Association) wax between the two pieces. These were then screwed together and the wax which extruded was pared away. The photometer has not recently been used at sea, but has been, since December, 1929, upon the roof of the Laboratory attached to a Cambridge Inst. Co. "thread recorder" (1930, 1). No leakage into the cell has occurred.

(*b*) The Neon Lamp Method.

This has been described by J. H. J. Poole (1928), who first devised it, and is figured by J. H. J. and H. H. Poole (1930).

We are indebted to Dr. J. H. J. Poole and to the Physical and Optical Societies for permission to reproduce the figure (Fig. 2). The wooden box containing the condensers, neon tube and safety resistance R (about 20,000 ohms) is indicated in section. Its shape has been modified to

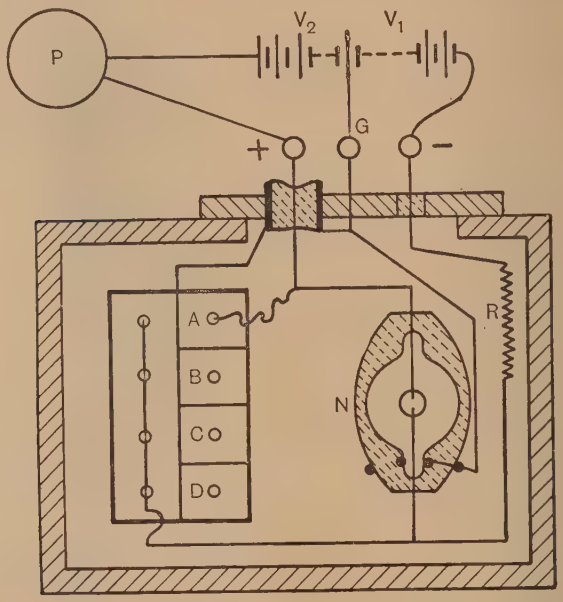

Fig 2.

show the electrical connexions more clearly. For a detailed description the original paper should be consulted.

The ordinary photo-electric circuit is used to charge a condenser, or, in practice, one of a set of four, of capacities as shown, A $=0{\cdot}5\ \mu$F, B $=0{\cdot}05\mu$F, C $=0{\cdot}005\mu$F and D $=0{\cdot}0005\mu$F, any one of which can be used by making connexion with a wander plug. A neon lamp, N, is connected in parallel with the condenser. Provided the voltage across the terminals of the lamp exceeds about 130 a flash will appear from time to time in it, the condenser being partially discharged thereby. It has been shown that the rate of flashing is proportional to the charging current, namely to the mean value of the illumination over the period. The voltage must be high enough not only to ensure flashing but also so that the effective

potential across the photo-electric cell, P, is always in a region in which small changes are without great effect upon the sensitivity, if absolute measurements of illumination are to be made. A sufficient potential, V_1, should be connected to a guard ring, G, attached to the neon lamp, to overcome the effect of small leaks, otherwise a current due to a certain number of metre candles would be required merely to balance the leakage. If the guard-ring potential be too high, flashing will occur in the dark. With a small rate of " dark " flashing, for which allowance can be made, the apparatus is in its most sensitive condition. Usually the total potential, V_1+V_2, should be 230–240 volts, and that across the guard-ring 170 volts.

In the measurements here described the ideal conditions were not satisfied, for though flashing began at about 132 volts, with photometer M, it was not possible to go much above 150 volts without obtaining " dark current " flashes, though at 60 volts M had previously showed no signs of leakage, internal or external. It had accordingly been fitted up without connecting its own guard-ring. For uniformity therefore a nominal, and closely an actual, voltage of 150 was selected and was employed with the deck photometer H (potassium vacuum) and the submarine photometers M (sodium vacuum), L (potassium vacuum) and J (potassium gas-filled).

These preliminary measurements with the neon lamp method were obviously unsuited for the accurate measurement of illumination, especially for low intensities, since the absence of a guard-ring potential introduced the error mentioned above, namely, that a certain minimum illumination was required to overcome the effects of leakage in the neon lamp and condensers. On the other hand, an error of the opposite sign was introduced by the leakage in photometer M. This error was much the larger of the two, and to keep the net dark-current down to a reasonable magnitude, which could be corrected for, the potential was reduced, as above. Hence the anode potential across the photo-electric cell was small, and its variation during the cycle of charge and partial discharge of the condenser must have considerably affected the sensitivity of the cell.

The interest lies rather in determinations of a comparative nature near the surface or at a small number of metres below it. These were, where possible, checked against determinations made by the potentiometer,

It must be pointed out that the effective capacity may differ considerably from the nominal capacity in parallel with the neon lamp, for the leads themselves have a certain capacity which may be quite considerable in the case of the submarine cable. This lessens the sensitivity of the method for work in the sea, but does not impair its usefulness for the lesser depths. This compounded capacity may be denoted thus : A M, capacity A ($0{\cdot}5\mu$F) with that of photometer M, including its leads. In

practice the time taken for a convenient number of complete flashes is measured; from this we can at once find either the time (t) of a single flash, which is inversely proportional to the current, or the number of flashes per minute (n) which is directly proportional to the current.

Inspection of Table I will make the method of standardization clear. This work took one hour under conditions of steady illumination and would have taken longer with variable light or at sea.

TABLE I.

September 29th, 1929. Standardization of neon lamp apparatus on roof of Laboratory, Plymouth, with photometers H and M side by side on parapet. H was used with potentiometer to determine V, shown in thousands of metre candles, k.m.c. When H was attached to the neon lamp apparatus, M, previously standardized against H, was used with the potentiometer. Number of flashes per minute=n. The window of M was wet as used at sea. Voltage was 150 on neon lamp apparatus, usual 60 v. with potentiometer. The series given are the means of five, four, five, and six highly concordant determinations respectively.

G.M.T.	V., k.m.c.	nAM	V., k.m.c., per flash. per min.	Ratios of sensitivities. $\frac{AM}{AH}$
3.10–3.24	40·58	17·6	2·31	11·0
		nAH		$\frac{BH}{AH}$
3.25–3.46	35·18	1·38	25·50	12·4
		nBH		$\frac{BM}{BH}$
3.47–3.59	32·20	15·7	2·06	8·2
		nBM		$\frac{BM}{AM}$
4.0–4.10	27·35	108·9	0·251	9·2

Next day, September 30th, the apparatus was taken to sea on the *Salpa*, but the weather was so bad that preliminary trials only could be carried out, inside the Breakwater. It was necessary to ascertain whether the capacity of the cables attached to M was altered by immersion. The coil had never been in salt water before. The wind was from S.W., strong and freshening, waves breaking, uniformly grey sky.

The ratio of the rates of flashing were determined with photometers side by side: (*a*) coil in air, (*b*) half in water, (*c*) almost all in water; results were obtained respectively for this ratio:—BM/BH, (*a*) 7·53, (*b*) 5·75, (*c*) 7·09, and 7·47. Under the adverse weather conditions these may be taken to indicate no change. A small alteration would not seriously affect comparative results. Taking the mean of the ratios, omitting the second, we see that BM/BH=7·35, as against 8·22 on the roof the previous day, using the same method and voltage. Comparing M and H, each with 60 volts anode potential, by means of the potentiometer, the ratio M/H=8·89 was obtained as against 8·75 under good weather conditions on the Laboratory roof on September 27th. This ratio 8·89 gives 4·75 metre candles per 10^{-9} amp. for M, for diffuse light, the corresponding value for H being taken as 42·3 m.c. It may be pointed out that the M H

ratios determined by the potentiometer and neon lamp methods are not comparable, both on account of the different voltages -the effective voltage in the latter being low—and of the capacity effect.

TABLE II.

V_a and V denote the illuminations, in k.m.c., as shown by the air and submarine photometers; ss denotes photometers side by side on deckhouse roof, *a* denotes submarine photometer swinging just clear of the water and *b* just below the water surface. The percentages p and p' refer to the roof and sub-surface positions respectively, p_n and $p_{n'}$ being the corresponding values found with the neon lamp apparatus. The values for the vertical absorption coefficients μ_v‡ and μ_{vn} (the latter with the lamp), are entered opposite the lower depth of the range taken, viz. 5 m. value is 4 to 5 m.

Time of flash, with 0·05μF condenser=2·70 sec., for photometer H and 0·394 sec., for photometer M, ratio M/H=7·71, at 2·11 G.M.T.

Date, Remarks, etc.	G.M.T.	V_a k.m.c.	*d* m.	V k.m.c.	p	p'	μ_v	p_n	$p_{n'}$	μ_{vn}
SERIES 47 and SERIES N2 2.10.'29.	2.4	40·2	ss	40·2	100	121	—	100	138	—
Off Pier Cellars, Cawsand Bay, depth 8 m.	2·34	40·4	b	33·3	82·5	100	—	72·7 71·8	100	—
	2.42	35·8	1	13·0	36·3	44·1	0·821	—	—	—
Wind strong N.W., waves	2.51	60·4	1	21·1	35·0	42·5	0·858	—	—	—
breaking at times, long	2.58	56·9	2	10·6	18·6	22·6	0·630	19·0	26·3	0·867†
swell. Sun weak through	3.25	19·5	3	1·62	8·29	10·1	0·809	10·7	14·8	0·568
broken clouds, some blue	3.46	25·9	4	1·04	4·01	4·87	0·725	4·74	6·56	0·821
sky.	3.50	20·2	5	0·385	1·91	2·32	0·744*	1·79	2·48	0·969
	4.4	18·7	6	0·236	1·26	1·52	0·420	0·49	0·68	1·31

* Mean 0–5 m., 0·746 for μ_v and 0·818 for μ_{vn}.

† 0 – 2 m.

‡ In previous papers we used μ for the absorption coefficient calculated on the supposed mean length of the path of the beam reaching the photometer and λ for the vertical absorption coefficient. μ_v is now used for the vertical absorption coefficient as when dealing with the colour of the light we require the usual symbol λ to denote wave-length.

Date, Remarks, etc.	G.M.T.	α°	Light.	β	*d* m.	V_a k.m.c.	V k.m.c.	p %	p' %	μ_v
SERIES 48. 14.10.'29.	1.25		Sun clear, sky	2·32	—	52·0	52·0	100	134	—
M Photometer. At E1.	1.36		clear blue, few		a	51·1	45·2	88·5	116	—
Wind S.W., very light,	1.45		clouds low on		b	51·9	38·8	74·8	100	—
water smooth, almost	1·50	31	horizon. Visi-		1	51·9	32·2	62·0	82·9	0·188
glassy, only slight swell.	1·56		bility good.		2	45·9	20·4	44·5	59·5	0·332
Depth 73 m.	2.0				5	45·7	9·91	21·6	28·9	0·238
	2.4	30			10	44·2	2·70	6·11	8·18	0·253
	2·7				15	44·0	1·02	2·32	3·10	0·194
	2.9				20	43·6	0·396	0·91	1·22	0·187
	2·12	29			25	43·6	0·163	0·375	0·502	0·177
	2·16				30	43·1	0·068	0·157	0·210	0·174
	2·19	28			35	43·1	0·029	0·066	0·088	0·172
	2·25	27			40	37·9	0·010	0·026	0·035	0·186
	2·39				35	38·4	0·017	0·045	0·060	0·168
	2·42	25			30	38·4	0·052	0·131	0·175	0·219
	*									
SERIES 49. 14.10.'29.	4.6	12		1·21	—	14·4	14·4	100	—	
M Photometer. At E1.	4·12	11			5	13·9	2·94	21·1	28·2‡	0·252‡
	4·16				10	13·5	0·878	6·50	8·7	0·236
	4·21	10			15	11·9	0·258	3·07	4·1	0·150
SERIES 50. 14.10.'29.	4·33	8	Sun clear, but		5	9·54	1·410	14·80	37·2	0·198
L Photometer. At E1.	4·36		low, sky clear,		10	8·90	0·444	4·99	12·5	0·217
Surface glassy, a flat	4.40	7	visibility good.		15	8·52	0·134	1·56	3·9	0·232
calm. Secchi disc 8½ m.	4·42			1·11	—	7·74	—	—	—	—
in sun on W., at 4.45 and	4·44	6		1 08	—	7·36	—	—	—	—
9 m. in shade on E.	4·57	4			b	5·17	2·055	39·8	100	—
	5·1	3			a	4·45	2·120	47·7	123	—

* The neon lamp method series N3 came in between.

‡ Calculated on the sub-surface value 74·8 per cent of Series 48, or allowing the usual 15 per cent loss only 0·278.

Date, Remarks, etc.	G.M.T.	$\alpha°$	Light.	d m.	V_a k.m.c.	V k.m.c.	$\frac{p}{\%}$	μ_v
Series 51. 15.10.'29.	12·6	31	Sun hazy but	5	43·1	8·56	19·8	0·291
M Photometer. Near	12.11		clearing, sky	10	44·3	2·36	5·32	0·263
El. Wind E., light.	12·19		blue with much	10	64·8	3·23	4·97	0·276
Secchi disc 10 m. in	12·29		haze.	15	58·1	1·02	1·77	0·207
shadow of ship to W.,	12·34			20	57·0	0·386	0·678	0·191
9 m. in sun on E. of ship.	12.39			25	56·2	0·161	0·286	0·172
	12·44			30	56·2	0·060	0·107	0·197
	12·48			35	57·9	0·025	0·043	0·181
	12·52			5	59·2	9·05	15·2	0·330*
	1·0	30		ss	56·2	56·2	100·0	—

Date, Remarks, etc.	G.M.T.	$\alpha°$	Light.	d m.	H k.m.c.	p_h‡ %	$\frac{p_h}{p_v}$	μ_h
Series 52. 15.10.'29.	1.21	29	Sun clear, visi-	5	51·4	2·700	5·26	0·266	—
M Photometer. Slung	1.25		bility good, no	10	51·7	0·995	1·93	0·375	0·201
horizontally. Near El.	1.28		clouds.	15	50·9	0·248	0·487	0·276	0·275
Moderate swell, waves	1·31	28		20	50·4	0·121	0·241	0·356	0·140
breaking, wind S.E., light	1.33			25	48·7	0·045	0·093	0·323	0·191
to moderate.	1.36			30	48·7	0·034	0·069	0·650	0·058
	1·40	27		5	48·5	1·770	3·66	0·184	— †

* 5–35 m., $\mu_v = 0{\cdot}195$.
‡ Relative to the vertical light in air, p_v is p of Series 51.
† 5–30 m., $\mu_h = 0{\cdot}158$.

Date, Remarks, etc.	G.M.T.	$\alpha°$	Light.	d m.	V_a k.m.c.	V k.m.c.	$\frac{p}{\%}$	μ_v
Series 53. 15.10.'29.	2.42	22	Sun clear, low	ss	38·4	38·4	100·0	—
Photometer J. Near	2.45		clouds only,	ss	36·7	36·7	100·0	—
El. Wind S.E., light to	2.57		visibility good.	5	32·2	6·57	20·4	0·285*
moderate. swell moderate	3.5	20	$\beta = 1{\cdot}59$.	10	31·2	2·02	6·50	0·228
and decreasing.	3.10	19		—	28·9	—	—	—
	3.15	18		15	26·7	0·644	2·41	0·198
	3.19			20	26·8	0·330	3·12	0·134
	3.22	17		25	26·3	0·187	0·712	0·110
	3.32	16		30	23·6	0·110	0·467	0·084
	3.35			35	23·6	0·073	0·310	0·082
	3·44	14		40	20·0	0·047	0·236	0·056
	3.55	13		35	15·1	0·048	0·317	0·078
	3.59			45	14·9	0·022	0·149	0·091
	4·5	12		50	14·3	0·011	0·075	0·073†
	4.12	11		55	10·8	0·008	0·072	0·114‡
	4.19	10		5	8·88	1·90	21·4	0·275*
	4.26	9		5	7·53	1·63	21·7	0·272*
	4.34	8		ss	6·51	6·51	100·0	—

* Assuming 15% loss at surface, including shading due to ship.
† For 45–55 m. ‡ For 5–55 m.

Date, Remarks, etc.	G.M.T.	$\alpha°$	Light.	β	d m.	V_a k.m.c.	V k.m.c.	$\frac{p}{\%}$	μ_v
Series 54. 21.10.'29.	12.10	29	Sun and blue	2·23	—	48·4			
Photometer J. About	1.12	28	sky with mov-			42·8			
one mile W. of Rame Hd.,	1.26		ing clouds.			21·4			
depth 33 m. Wind S.W.,	1·30					23·9			
light to fresh, some waves	1.42				ss	45·3			
breaking.	1.48				ss	47·6			
	1.54				ss	21·6			
	1.59	24			ss	18·9			
	2.7				ss	40·4			
	2.25	22		1·82	5	33·1	5·78	17·5	0·315
	2.33	21			10	33·6	1·46	4·35	0·278
	2·45	20			15‡	27·4	0·406	1·48	0·216
	2.57				15§	27·9	0·397	1·42	0·223
	2.59				15	25·5	0·376	1·48	0·216
	3.4	18			20	25·1	0·099	0·39	0·263
	3·8				25	24·6	0·036	0·15	0·199
	3.14				30	24·1	0·008	0·03	0·287*
	3·16	16		1·36	—	23·9	—	—	—
	3·30	14			5	18·9	2·89	15·3	0·342
	3.40	13			5†	15·1	2·16	14·3	0·356

‡ J at 12 volts up to this.
§ J at 60 volts. $J_{60}/J_{12} = 2{\cdot}34$.
† J at 12 volts again.
* 5–30 m., 0·244.
ss Photometers side by side for calibration.

Date, Remarks, etc.	G.M.T.	Light.	d	p	p'	μ_v
Series N1. 30.9.'29.	11.40	Sky overcast.	ss	100	168	—
Photometer M. Just inside Plymouth Breakwater, depth 13 m. Wind S.W., strong and freshening, waves breaking.		Thickening for rain, occasional drops in afternoon. At 12.30 V=18·6 k.m.c., and at 2.45 V=6·1.	a	70·5	118	—
			b	59·5	100	—
			b^1	55·5	93·5	—
			1	39·6	66·6	0·404
			2	25·6	43·1	0·435
			3	16·0	26·9	0·495
			4	10·0	16·8	0·469
	1.40		5	6·67	11·2	0·401
	2.0		6*	5·12	8·61	0·266
			7	3·43	5·77	0·399
			0–7	—	—	0·410

* Changed over from condenser B (0·05μF) to C (0·005μF) for each photometer, viz. H on deck-house roof and M in water.

Series N2. 2.10.'29.	Blue sky and broken clouds; sun weak through clouds.	ss	100	138	
Photometer M. Off Pier Cellars, Cawsand Bay, depth 8 m. Wind strong, N.W., long swell, waves breaking.		b	72·7	100	
		b^1	71·8	98·7	
		2	19·0	26·1	0·867
		2	19·1	26·2	—
		3	10·9	14·7	0·568
		3	10·5	14·3	—
		4	4·75	6·52	0·821
		4	4·74	6·51	—
		5	1·79	2·47	0·969
		6	0·49	0·67	1·31
		6	0·48	0·66	—
		0–6			0·831

Date, Remarks, etc.	G.M.T.	$\alpha°$	Light.	d	p	p'	μ_v
Series N3. 14.10.'29.	3.24	17	Sun clear, sky clear blue, few clouds low on horizon. Visibility good.	5	33·7	36·4	0·216
Photometer M. At E1, depth 72 m. Wind S.W., very light, water almost glassy, only slight swell.	3.29			4	41·8	45·2	0·144
	3.33	16		3	48·3	52·2	0·304
	3.39	15		2	65·5	70·8	0·138
	3.44	14		1	75·2	81·3	0·207
	3.51	13		b	92·5	100	—
	3.57			a	100	108	—
				0–5	—	—	0·202
Series N4. 21.10.'29.	3.52	11	Sun and blue sky with clouds, light fairly steady.	5	14·3	18·7	0·407
Photometer J. About one mile W. of Rame Hd., depth 33 m. Wind light to fresh, some waves breaking.	3.58	10		4	21·5	28·4	0·228
	4.6			3	27·1	35·7	0·356
	4.10	9		2	38·7	51·0	0·179
	4.16	8		1	46·3	61·0	0·392
	4.21	7		b	68·5	90·3	—
	4·29	6		a	77·0	—	—
	4.39	5		a	76·0	100	—
	4.50	3		ss	100·0	132	—
				0–5			0·312

DISCUSSION OF RESULTS.

Table II records the results obtained in Series 47–54, in which measurements were made as usual by the potentiometer method, and in Series N1–N4, in which the neon lamp method was used.

The lettered sections which follow relate in general to those similarly lettered in the introduction.

(*a*) It was never found possible to use the new vacuum sodium photometer (M) down to the bottom, 70 m.; the greatest depth was 40 m. (Series 48), at which the illumination was 10 m.c. Photometer L had previously been used to 40 m. (Series 19), the illumination being 150 m.c. The inadequate cable length prevented its use at a greater depth. Were the aperture to be increased somewhat and the cable lengthened it

appears that a potassium cell could be used to greater depths than a sodium cell. Thus at Station E1 on 14/10/'29 the sodium cell gave, for the vertical absorption coefficient, μ_v, between 5 and 35 metres, the value 0·193 (Series 48), whereas next day the gas-filled potassium cell J gave 0·139 over the same range (Series 53). Obviously the shorter mean wave-length registered by the sodium cell is more heavily absorbed than is the rather longer radiation to which the potassium cell is sensitive ; for the wave-length sensitivity data see Figure 2, page 466, Vol. 15, this journal. Such a result is in keeping with the findings of Shelford (1928) for the upper 15 m. in the Puget Sound, below which the transmission of the light affecting the sodium cell was the greater. A comparison of photometers M (sodium) and L (potassium vacuum) on 14/10/'29 at E1 gave, from 0–15 m., μ 0·228 and 0·213 for M and 0·216 for L. This is close agreement, but it may be seen in the figure that this particular potassium cell showed greater response to shorter wave-lengths than did other potassium cells ; in any case the gas-filled cells show a sensitivity shift towards longer wave-lengths. The three values of μ_v for 14/10/'29 were obtained at 2 P.M., 4.15 P.M. and 4.35 P.M., mean times, of each series, and serve to show that there was no appreciable alteration in absorption due to phototropic movements of zooplankton, as had been suspected previously on other occasions ; at this season, however, no considerable amounts of zooplankton would be found.

As regards the state of the water from year to year it is of interest to consider the mean values of μ_v from 5–35 m., at E1. The determinations in 1925, 1927 and 1929 were made with potassium gas-filled cells K and J, that for 1928 with a vacuum cell, L ; μ_v=0·146 (1/10/'25), 0·104 (3/10/'27), 0·124 (2/10/'28) and 0·139 (15/10/'29). At this time of year the vertical circulation, brought about by surface cooling, has just taken place, so that the water column is isothermal and homogeneous. There are, however, considerable differences in μ_v from year to year. These are probably to be sought in the amount of suspended matter, in which inshore water is richer than that further out to sea. Examples illustrating this may be found by comparing Series 47, Cawsand Bay, μ_v=0·78, Series N1, Breakwater, μ_v=0·41, Series 54, near Rame Head, μ_v=0·24, Series 51, near E1, but closer to shore, μ_v=0·19. These measurements were with the sodium photo-electric cell, save Series 54, in which a gas-filled potassium cell was used, giving a value rather lower than with a sodium cell. Since it was not possible, as shown in our 1929 paper, to correlate the changes in μ_v with seasonal changes it would appear desirable to study it on a line across the English Channel, or further out into the Atlantic, for it might give indications of the origin of the water similar to those given by salinity determinations, and perhaps more delicate, even though of lesser percentage accuracy.

(*b*) As previously pointed out the shadow error, due to taking the deck-house roof reading as being a measure of the illumination just above the water surface, is both large and variable. Thus in Series N1, with no sun, the shadow loss was 29·5 per cent of the total illumination as measured on the roof; in Series N4, with a clear sky and low sun it was 23·5 per cent; in Series 48, with a clear sun and sky and $\beta=2{\cdot}3$, the loss was only 11·5 per cent. In Series 50, with a clear sun and sky, but late in the afternoon with $\beta=1{\cdot}04$, the loss was 52·3 per cent; in this case the shading must have been abnormally great, the deck-house photometer perhaps sloped towards the sun with the ship's roll and the photometer above the sea was entirely shaded from the sun; but whatever the explanation such measurements were obtained.

In obtaining illuminations below the surface the readings must be multiplied by the factor 0·935, which is the ratio for the light in air to the light just below a smooth water surface for diffuse daylight (see p. 184, 1926 paper).

The submarine photometers were standardized in air, with opal glass in position and window wet, bubbles being excluded. The light in air was measured by the roof photometer, H, so that in the water over the window may be taken as 0·935 of this. Accordingly the "air" reading of the submarine photometer corresponds to an illumination in the layer of water just outside its window only 0·935 times what one assumes for the purpose of standardisation. The factor is an approximate one for an assumed uniformly bright sky. This correction has been made in obtaining the figures given in the table.

The light lost upon entering the water was measured directly in five series, being obtained from the illumination just above the surface "*a*" in the tables, and that just below the surface "*b*." Values for $a-b$ were as follows: Series N1, 15·5 per cent; N3, 7·5; N4, 10·5; 48, 15·5; 50, 16·5; mean, 13·1.

Formerly, in calculating μ_v, 0—5 m., we took the deck-house roof illumination and deducted 15 per cent to obtain the sub-surface illumination, *b*. This would have been approximately correct for the surface loss, but is quite inadequate for the shadow loss.

It would therefore appear that it is essential to obtain *a* directly if *b* cannot be obtained on account of the roughness of the sea. If neither can be obtained values for the percentage submarine illumination may be got by assuming a surface loss of 15 per cent for choppy water or 8 per cent for calm water, and making an approximate allowance for the shading due to the ship according to the lighting conditions prevailing at the time.

It follows from this that the neon lamp method is of value, for only under really good weather conditions can *a* and *b* be obtained by the

potentiometer method. The latter, however, has the advantage for work down to the greater depths.

(*c*) As regards measurement of the "horizontal light," namely, that falling on a vertical surface, only one series (No. 52) has been obtained in addition to those previously reported, Series 15 (12/9/'27), Series 25 (6/3/'28). The latter series, with an efficient opal diffusing disc, gave 0·61 — 0·47, mean 0·54 as the ratio of horizontal to vertical light from 0 — 25 m. Series 52, within the same limits, gives 0·37 — 0·22, mean 0·31, as determined with sodium photometer, instead of potassium in Series 25. Further measurements are desirable.

SUMMARY.

1. J. H. J. Poole's neon lamp method (1928) of integrating the photo-electric current is serviceable for work at sea down to moderate depths, which further experience may extend considerably. It is specially valuable for determining the light just above the water, and at such depths down to 5 m., at which the variability of the light renders the potentiometer method (1925) very difficult or quite impossible in rough water. For greater depths, down to 70 m. (bottom in the English Channel around our normal range) we have so far been able to use the latter only.

2. The loss due to the shadow of the ship, obtained by subtracting the illumination just above water from that on the deck-house roof, was found to vary from an extreme case of 52 per cent, and a normal loss of 30 per cent, with an overcast sky, down to 11 per cent with a clear sun at 31° altitude.

3. The loss of light due to its entering the water was found to vary from 7·5 to 16·5 per cent, mean 13·1 per cent.

4. It is desirable that the illumination just above and just below the water surface should be determined by the neon lamp method as a routine.

5. It was found that, under the isothermal and homogenous water conditions obtaining during the first half of October, the vertical absorption coefficient, μ_v varied at Station E1 (about twenty miles outside Plymouth Sound) from $\mu_v = 0{\cdot}146$ in 1925 to $\mu_v = 0{\cdot}104$ in 1927, 1928 and 1929 being intermediate with $\mu_v = 0{\cdot}124$ and $0{\cdot}139$ respectively, over the range 5–35 m. These potassium cell values are less than similar determinations with a sodium cell, for which the value $\mu_v = 0{\cdot}193$ corresponds to the 0·139 value for potassium.

6. A modified form of submarine photometer case has been described, so constructed that its opal receiving surface can be altered from the

horizontal to the vertical or inverted positions merely by altering one or two bolts and shackles. It has two shackles instead of four. The one series of measurements made with it and its sodium cell, gave a mean value 0·31 for the ratio of the horizontal to the vertical submarine illumination between 0 and 25 metres.

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The Effect of Light of Different Intensities, Reduced Selectively and Non-selectively, upon the Rate of Growth of *Nitzschia closterium.*

By

F. A. Stanbury, M.Sc.

With 7 Figures in the Text.

Introduction.

The effect of light upon plant growth is a problem which has attracted many workers since the discovery of Priestly, in 1771, that a plant could purify fixed air, carbon dioxide, which was followed by the supplementary work of Ingen-Housz and Senebier, who showed that the phenomenon was associated with the nourishment of the plant, and took place only in sunlight and through the agency of the green portions of the plant.

Senebier, 1782, was perhaps the first to attempt to show the effect of the different parts of the spectrum on the process, and found that red light was almost as active as ordinary light in the process of photosynthesis whilst the blue was almost inactive. Draper and Daubeny, 1844, concluded that after white light, the red-yellow portion possessed the highest efficiency, and this was supported by Sachs in 1868. Englemann, Timiriazeff and Wolkaff, however, found that whilst the activity in the red was high, there was an important secondary maximum of efficiency in the blue, and these results have been subject to great controversy amongst later workers.

It must be borne in mind that the physics of the light used by the early investigators was not thoroughly understood, and one of the greatest criticisms brought against these workers is that the blue light used by them contained parasitic rays of long wave lengths, viz. in the red region. Dangeard (1927) asserts that in light of feeble intensity, these parasitic rays are practically inactive, but in light of greater intensity they play a highly important role, and are the cause of the secondary maximum observed by many workers. Richter (1902) using coloured screens, concluded that the rate of photosynthesis was proportional to the amount of energy absorbed, independent of the part of the spectrum and the wave length of the ray, and Kniep and Minder (1909) determined that the

action of the red and the blue was almost similar when the energy in those regions was the same.

Klugh (1925) sought to determine whether photosynthesis was a wave length phenomenon, or dependent upon the total light intensity. To do this he grew unicellular algæ, in light which he regarded to be of the same intensity but of different wave lengths, and took the rate of reproduction as criteria for efficiency of the wave lengths concerned in photosynthesis, for although the method was indirect it was assumed that, fundamentally, the rate of reproduction in chlorophyllous organisms is dependent on the ability to manufacture food. From a single series of experiments lasting from August 16th–September 10th he concluded that photosynthesis is a wave length phenomenon, red light being highly efficient, blue much less so, and green inefficient, but Klugh himself in his concluding remarks says "that it is dangerous to draw conclusions from a single series of experiments."

Using somewhat similar methods to those of Klugh, results are now presented in this paper, which would show that photosynthesis, as indicated by the rate of growth of marine diatoms, is a function of the amount of energy transmitted independent of the wave length between which the energy so transmitted lies.

Plant material used.

Persistent cultures of the marine diatom *Nitzschia closterium*, grown in "Miquel sea water," were used for the plant growths to be studied, a persistent culture, as defined by Allen and Nelson (1910), being one in which only one species of diatom was present, although there might be bacteria.

It is of interest to mention here, that marine diatoms exhibit well-marked periodicity in the sea, the diatom outburst being at its height between early March and late April. The cause of these outbursts is not fully understood.

Marshall and Orr (1928) concluded that the length of day was a factor of the greatest importance, whilst Atkins, Herdman, Scott and Dakin correlated the early spring sunshine with the diatom outburst. Atkins (1928) also found that "a study of the phosphate change affords a measure, in an inverse ratio, of the production of the algal crop, and indicates from year to year the variations that occur in its seasonal waxing and waning."

From results obtained using the marine diatom *Nitzschia closterium* it would appear that the total light intensity is a factor which has a profound effect upon growth.

Method.

It was desired to determine the effect of light of selected wave lengths and of daylight reduced in intensity non-selectively, upon the rate of growth of *Nitzschia closterium*, and this was done by growing cultures of Nitzschia under a series of selective and non-selective filters.

Equal volumes of "Miquel sea water," contained in sterilized crucibles of white porcelain of 10 cubic centimetres capacity and 3·5 centimetres

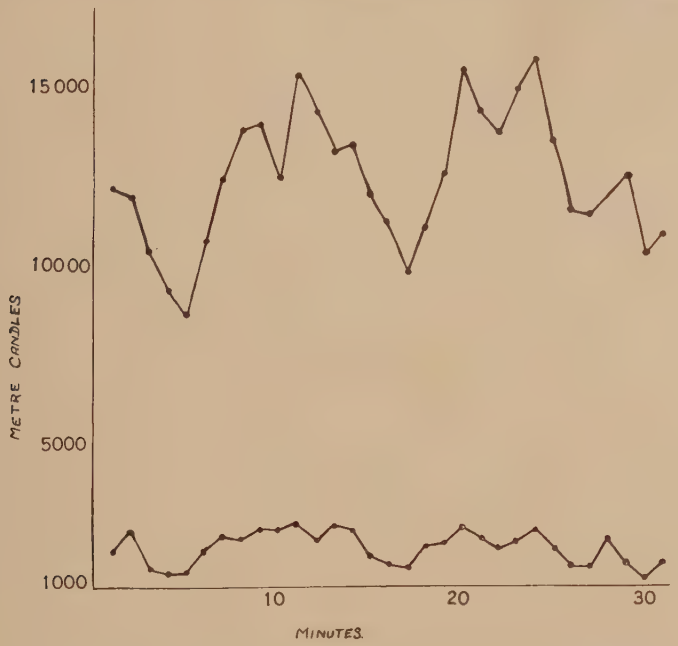

Fig. 1.—Curves showing the variations in illumination for the north window, lower curve, where the cultures were set, and for an open site on the laboratory wall, upper curve, determined simultaneously for a period of 30 minutes. The numbers along the ordinates represent the illumination in metre candles. Those along the abscissæ the time in minutes.

diameter, were inoculated with similar drops of a persistent culture solution, which contained known numbers of diatoms per unit of volume.

The number of diatoms in any culture was determined with the aid of a hœmacytometer, the millimetre square being ruled in Thoma pattern. The culture to be examined was first thoroughly mixed by means of a fine sterilized pipette, this process also serving to aërate the culture. A drop of the mixture was then placed upon the hœmacytometer, and the number of diatom cells per unit of volume, 0·1 cubic millimetre, was then

found by counting. For each reading an average of four counts was made, a difference of ten diatoms being allowed for any accepted count.

Examination of the tables of results as a whole, will show that it would have been better to have used larger initial number of diatom cells since their minute size renders them very difficult to count when present in small numbers. This explains the irregularities in the early counts for the cultures of each series.

The sub-cultures were covered with the selected filters and then placed side by side in the window of a north room. The distance between the two end cultures was 80 centimetres. Opposite the window and 4·2 metres away from it, is a high grey wall of limestone, which receives direct sunshine only for a short time as the sun sets.

On 27.2.30, simultaneous measurements were made of the illumination of the window in the north room (V_s) and of a perfectly open site on the laboratory roof (V_o) by means of photometers furnished with vacuum photo-electric cells. (For method see Atkins and Poole, 1929.) It has been shown that on a dull day, the illumination at a point inside a building bears a very nearly constant ratio to the illumination at a point outside, and this ratio, expressed as a percentage, has been termed the daylight factor δ. Table I shows these values over a period of half an hour.

TABLE I.

February 27, 1930. Grey sky, completely overcast, wind N.E. 12.14–12.43 p.m. G.M.T.

Indoor site is the window-sill of a north room, where the photometer is moved over a range of 80 cm. The outdoor site is a fixed position on a parapet of the Laboratory roof.

Indoor sites (in window sill).		G.M.T.	Illumination in meter candles. In open on Roof Vo.	In window sill Vs.	Daylight Factor. $\frac{Vs.}{Vo.} = \delta$	Average δ
Site 1.	1.	12.14	12,000	1,950	16·2	
Right extremity	2.	12·16	10,200	1,490	14·6	
occupied by	3.	12·17	9,150	1,389	15·2	
cultures.	4.	12·18	8,500	1,392	16·4	
						15·6
Site 2.	1.	12·19	10,500	1,930	18·5	
To right of centre.	2.	12·20	12,200	2,360	19·3	
	3.	12·21	13,600	2,340	17·2	
	4.	12·22	13,800	2,520	18·3	
						18·3

Indoor sites (in window sill).		G.M.T.	Illumination in meter candles. In open on Roof Vo.	In window sill Vs.	Daylight Factor: $\frac{Vs.}{Vo.}=\delta$	Average δ
Site 3.	1.	12·23	12,200	2,520	20·7	
Centre of window.	2.	12·24	15,200	2,680	17·6	
	3.	12·25	14,100	2,290	16·2	
	4.	12·26	13,000	2,680	20·6	
	5.	12·27	13,200	2,560	19·7	
	6.	12·28	11,800	1,880	15·9	
						18·45
Site 4.	1.	12·29	11,100	1,610	14·5	
To left of centre.	2.	12·30	9,690	1,410	14·7	
	3.	12·31	10,900	2,110	19·3	
	4.	12·32	12,400	2,170	17·5	
	5.	12·33	15,400	2,660	17·4	
	6.	12·34	14,200	2,310	16·3	
						16·6
Site 5.	1.	12·35	13,600	2,060	15·2	
Extreme left of	2.	12·36	14,800	2,260	15·3	
centre.	3.	12·37	15,600	2,660	17·1	
	4.	12·38	13,300	2,000	15·1	
	5.	12·39	11,400	1,610	14·2	
	6.	12·40	11,300	1,550	13·8	
	7.	12·41	14,900	2,310	15·6	
	8.	12·42	12,400	1,670	13·5	
	9.	12·44	10,800	1,680	15·6	
						15·6

Total average for the four sites is 16·6 for δ.

The Filters used.

The filters selected were twelve in number, and Table II gives the details concerning their transmissions of energy and wave length. The non-selective screens were Wratten filters of certain known reduced intensities. The transmissions (T) for these were :—T=50%, 25%, 12·5%, 6·25% and 3·1% respectively, but unhappily the gelatine films were damaged at the outset of the experiments, and their actual transmissions determined photo-electrically, using a sodium vacuum sensitive cell, were T=41·8%, 19·2%, 9·24%, 3·26% and 1·66% respectively.

For convenience these filters will be referred to as Wratten filters T=$\frac{1}{2}$, T=$\frac{1}{4}$, T=$\frac{1}{8}$, T=$\frac{1}{16}$ and T=$\frac{1}{32}$ respectively. The makers' figures refer, however, to light incident normally whereas the photo-electric

measurements refer to light received from the sky as a whole. The latter are necessarily lower than the former on account of the oblique incidence.

Six selective filters were used. Four were of Corning glass of red, orange, green and blue shades, and the actual percentage transmissions for these were determined by Dr. H. H. Poole (Dublin) by means of a Moll thermopile. Figure 2 shows the curves obtained from his data together with the transmission curves of the other coloured filters used.

TABLE II.

To show the wave lengths, light transmissions and relative energy values for the screens and filters used for the culture experiments.

Screen or filter used.	Thickness of screen used. mm.	Wave length in mμ.	Actual percentage light transmitted T.	Average relative energy in arbitrary units (from Dr. Abbot's data).	Total percentage relative energy transmitted E.
Solar spectrum			100	2,880	100
Wratten neutral					
1. Wratten T=$\frac{1}{2}$		400–720	41·8	1,240	41·8
2. ,, T=$\frac{1}{4}$		,,	19·2	570	19·2
3. ,, T=$\frac{1}{8}$		,,	9·5	274	9·5
4. ,, T=$\frac{1}{16}$		,,	3·3	95	3·3
5. ,, T=$\frac{1}{32}$		,,	1·7	49	1·7
6. Ordinary glass		,,	71·0	2,040	71·0
7. Corning red	2.96	620–720	67·1	602	20·9
8. ,, orange	2·96	570–720	73·0	990	34·5
9. ,, green	3·47	490–570	13·4	96	3·3
10. ,, blue	2·96	400–490	60·4	490	17·0
11. Schott and Gen blue	2·21	400–490	50·3	406	14·0
12. Chance blue		400–490	36·2	293	10·2
13. Heat absorbing screen		400–720	27·7	795	27·7
14. Heat absorbing + Corning blue		400–490	16·7	135	4·7

One of these was a blue glass prepared by Schott and Gen of Germany, and the transmission curve was obtained from data supplied by them. The other blue filter was prepared by Chance of Birmingham and the curve for it was obtained by resolving values read from a photograph of the density curve supplied by them. $\left(\text{Density} = \log \frac{1}{\text{transmission}}\right)$.

It may be observed from Figure 2, that the Corning blue transmits a certain low percentage of parasitic red rays. These were eliminated in some experiments, by placing a heat absorbing screen of a green shade over the Corning blue, which effectively cuts off these red rays, but lowers the intensity considerably. A suitable correction has been applied for this, in interpreting the results with the combination of screens. The

other blue screens furnish a blue light of a high degree of purity, the Chance blue shows no inclusion of red at all.

Lastly, a filter of ordinary glass was used as a control, and since it was of a greenish tinge the amount of blue light passing through was lowered, and the total intensity of the light received reduced.

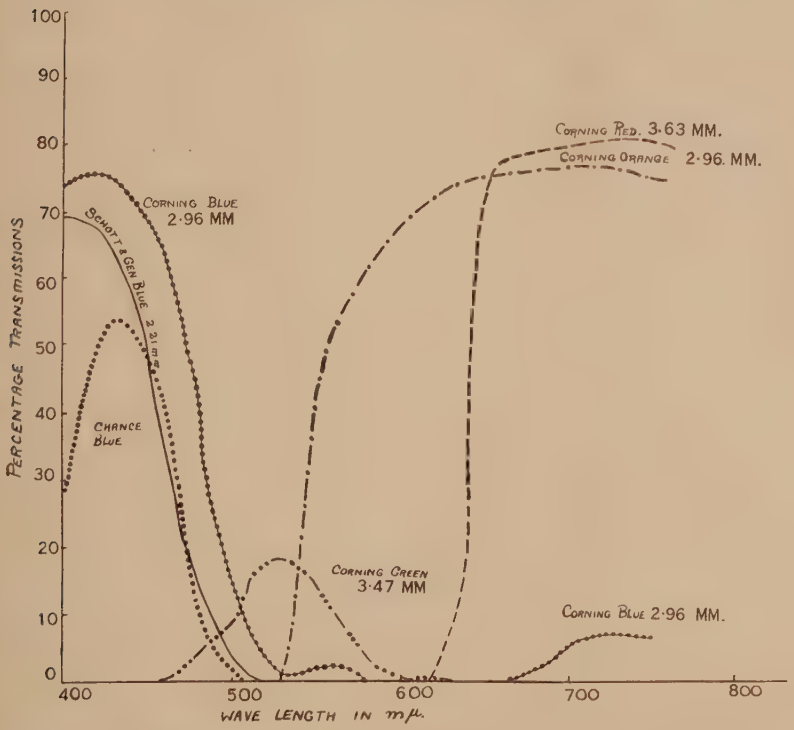

FIG. 2.—The ordinates are percentage transmissions for the selective screens used as determined, using a Moll thermopile. The abscissæ are wave length in mμ. The curves for the Chance blue, Schott and Gen blue glasses were obtained from data supplied by those firms as corrected for reflection losses for light incident normally.

The Relative Energy of the Filters Used.

Owing to lack of apparatus it was impossible to determine the relative energy transmitted through the screens directly, but it is hoped that this will be done shortly. The energy value is one which is undergoing constant change throughout the day, since it varies considerably in the different portions of the spectrum, and the proportion of the red, yellow, green and blue light changes from hour to hour according to the time of day and the condition of the sky light. An attempt to obtain approximate

values of the relative energy in arbitrary units was made in the following manner.

At the Seventh International Congress of Photography, 1929, it was recommended that the following energy values derived from data of Dr. C. G. Abbot (Smithsonian Institute, U.S.A.) should be adopted as defining the spectral composition of mean noon sunlight. Table III shows these values for the visible part of the spectrum.

TABLE III.

Relative energy values from data of Dr. C. G. Abbot.

Wave Length.	Relative Energy.	Wave Length.	Relative Energy.
360	16·0	550	101·7
370	20·5	560	100·0
380	25·1	570	98·4
390	30·1	580	97·2
400	45·2	590	95·6
410	57·2	600	95·2
420	65·8	610	94·3
430	69·2	620	93·2
440	77·2	630	92·2
450	86·8	640	91·0
460	92·2	650	89·7
470	96·9	660	88·5
480	99·0	670	86·4
490	100·6	680	84·7
500	101·8	690	82·7
510	101·2	700	80·5
520	101·2	710	78·1
530	101·1	720	76·1
540	100·9		

Using these figures it was calculated that the average value for the relative energy transmitted from 720mμ—400 mμ. was 89·9 units per sq. cm. per minute, and the light transmitted over this region was taken as 100%. The total energy transmitted, E, for the visible spectrum was thus proportional to $89{\cdot}9 \times (720-400) = 2880$ arbitrary units. Since this is the maximum value for E, it may be taken as 100, and the values of E for the light filters then found by substitution, e.g. considering the Corning red filter $\frac{89{\cdot}9 \times (72-62)}{2880} = 31{\cdot}2\%$, and this is the proportion of

red as defined in the spectral curve. Of this the filter transmits 67·1%, so that the total relative energy transmitted is only 21·0%. Column 5, Table II, shows the values of E for all the filters used.

RESULTS OF OBSERVATIONS

Attempts were first made to conduct the experiments in the open, but the temperature was too high for the growth of the diatoms, and the light intensity also, for all but the more heavily screened.

Three series of observations were made indoors :—a preliminary series in May, 1929, followed by a second series June 13th–July 28th, 1929, which is referred to as the "summer" series. In both cases filters 1–10, Table II, were used. During the interval between the second and third series (January–March, 1930), the actual transmissions of the filters had been determined by Dr. H. H. Poole, revealing the presence of parasitic red rays in the Corning blue. In the third set of observations, therefore, precautions were taken to exclude the red rays of the blue filter by placing a heat-absorbing screen over it, as previously described. The light intensity T was then reduced to 15·0 and the relative energy E to 4·7%. The Schott and Gen and Chance blue filters were used in January in addition to the other filters.

Preliminary Series, May 6th–28th, 1929.

Average hours of sunshine 9 hours daily.

Tables IV, V, and VI are the results of observations made upon the rate of growth of the marine diatom *Nitzschia closterium*, when grown in "Miquel sea-water," under selective or non-selective screens as described in the preceding pages. The following list of screens used indicates the order of the cultures in the window, starting from the right-hand side :—

Corning blue		(400 mμ–490 mμ)
,, green		(490 mμ–570 mμ)
,, orange		(570 mμ–720 mμ)
,, red		(620 mμ–720 mμ)
Ordinary glass		(400 mμ–720 mμ)
Wratten filter	T.=$\frac{1}{2}$ 41·8%	(400 mμ–720 mμ)
,, ,,	T.=$\frac{1}{4}$ 19·2%	,,
,, ,,	T.=$\frac{1}{8}$ 9·5%	,,
,, ,,	T.=$\frac{1}{16}$ 3·3%	,,
,, ,,	T.=$\frac{1}{32}$ 1·7%	,,

TABLE IV

SUB-CULTURES OF *Nitzschia closterium* GROWN IN CRUCIBLES UNDER ORDINARY GLASS, FROM 6TH–28TH MAY, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.
9.5.29	3	18
13.5.29	7	85
16.5.29	10	123
21.5.29	15	270
23.5.29	17	432
28.5.29	22	546

TABLE V.

SUB-CULTURES OF *Nitzschia closterium* GROWN IN CRUCIBLES UNDER NEUTRAL WRATTEN FILTERS FROM 9TH–28TH MAY, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.				
		T=$\frac{1}{3\cdot2}$	T=$\frac{1}{1\cdot0}$	T=$\frac{1}{8}$	T=$\frac{1}{4}$	T=$\frac{1}{2}$
13.5.29	4					
16.5.29	7	6	1	42	13	32
21.5.29	12	6	3	60	315	340
23.5.29	14	14	18	218	475	485
28.5.29	19	30	42	590	1400	1206

TABLE VI.

SUB-CULTURES OF *Nitzschia closterium* GROWN IN CRUCIBLES UNDER SELECTIVE FILTERS OF CORNING GLASS FROM 13TH–23RD MAY, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.			
		Red.	Orange.	Green.	Blue.
16.5.29	3	2	3	0	2
21.5.29	8	120	69	152	56
23.5.29	10	285	241	270	106

The figures in the tables represent the number of diatoms per unit of volume (0·1 mm.^{3}) as counted with the aid of a hæmacytometer. From these results it would appear during the period of observation for May 1929 :—

1. That the diatoms can utilise light of all wave lengths.

2. That light of reduced intensities is much more favourable than full illumination at this time of year. Light reduced to about 6%–3·3% being the most suitable of the series.

Second Series, June 13th–July 29th, 1929.

Table VII gives the results of observations from a new series of sub-cultures grown under the same conditions as in the preliminary experiments and occupying the same sites as formerly. During this period,

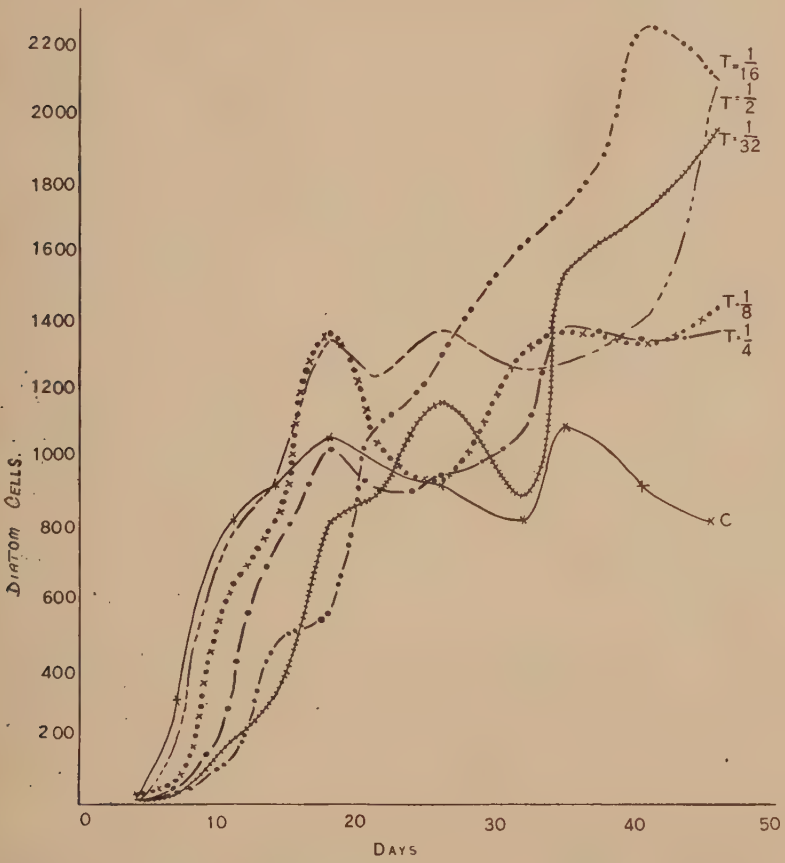

FIG. 3.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium*, when grown under screens reducing the light intensity non-selectively, for a period of 46 days during the months of June and July, 1929.

however, the average number of hours of sunshine was 8 hours 11 minutes for June and 8 hours 49 minutes for July. Many days had 14 hours sunlight. Figures 3 and 4 give the curves obtained from the data.

When grown in ordinary flasks, cultures of *Nitzschia closterium* appear yellowish brown. Distinct colour changes of the cultures grown under

the selective filters were observed during this second series, and also throughout the January–March growths. Under the heading of the concluding remarks for the last series, the subject is dealt with more fully. Reference to Figure 6 will show at a glance that a curious drop in the

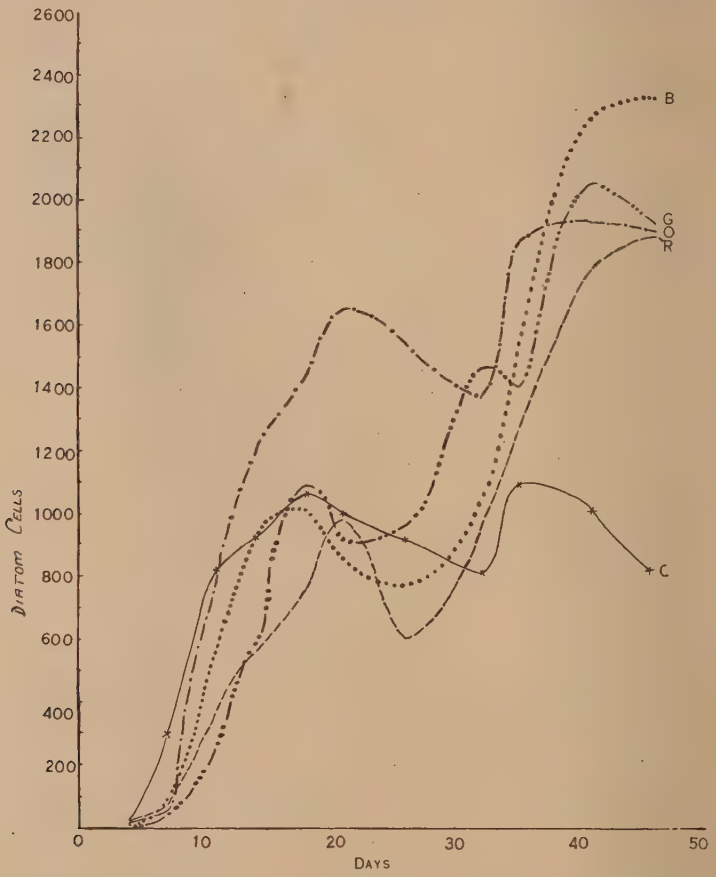

FIG. 4.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium*, when grown under screens reducing the light selectively, for a period of 46 intensity days during June and July, 1929.

number of diatoms is shown by all the growths. Allen and Nelson (1910) state that the siliceous shell of marine diatoms generally, is markedly thinner than in the great majority of other forms, and that this is even more emphasised in cultural forms. Dr. Allen has also stated that it is a common experience for cultures to show periodic decrease in numbers.

TABLE VII.

To Show the Growth of the sub-cultures of *Nitzschia closterium* Grown under Selective and Non-selective Filters, from June 13th–July 29th, 1929.

	No. of diatoms per unit of volume.									
	Wratten filters.					Ordinary		Corning filters.		
No. of days.	T=$\frac{1}{\frac{1}{2}}$	T=$\frac{1}{16}$	T=$\frac{1}{8}$	T=$\frac{1}{4}$	T=$\frac{1}{2}$	Glass.	Red.	Yellow.	Green.	Blue.
4 . .	4	4	24	8	2	15	19	20	9	8
7 . .	22	36	64	147	196	299	68	54	51	92
11 . .	184	132	620	336	792	816	384	786	276	576
14 . .	304	468	816	732	924	920	552	1184	584	920
18 . .	812	544	1360	1024	1344	1060	728	1444	1092	1016
21 . .	884	1072	1060	912	1234	996	976	1652	920	860
26 . .	1160	1296	932	940	1372	920	604	1548	968	772
32 . .	886	1624	1304	1088	1256	816	980	1372	1468	1060
35 . .	1544	1860	1368	1376	1720	1092	1276	1864	1404	1540
41 . .	1728	2256	1330	1340	1412	1012	1788	1928	2064	2272
46 . .	1868	2092	1432	1336	2080	812	1884	1900	1920	2328

Accordingly, a special watch was made for empty frustules, and these were very seldom seen. The problem of the fate of the diatoms, which causes these well-marked falls in the curves, is further discussed in the third series. It may be mentioned here, however, that this decrease can hardly be due to lack of food material. Under the ordinary course of the experiments the volume of the culture solutions was made up to 10 c.c. from time to time by the addition of distilled water, to compensate for the small losses in volume due to evaporation and the minute drops extracted for counting purposes. After the 29th July, 1929, however, the observations were abandoned for about two months. During this time the cultures remained untouched. Yet counts made for all the cultures on September 29th, 1929, showed such dense growths that it was almost impossible to see the ruling on the hæmacytometer when drops were examined in the usual way after the culture solutions had been restored to their original volume of 10 c.c. with distilled water.

Conclusions from Second Series.

Thus it appears that diatoms can utilise light of all wave lengths even in the green region of the spectrum. It is true that the Corning green transmits parasitic rays of yellow and blue, but the percentage is so small as to be scarcely responsible for the astonishing growth under a screen which has a total transmission of light of only 13·5%. Miquel (1892) found that orange light was best suited to the growth of freshwater diatoms. A study of Table VII will show that orange light is highly favourable for growth of the marine diatom Nitzschia, being slightly better than red, which it includes. During the last few days of the experiments the numbers under the blue and green filters exceeded those of the diatoms under both the red and orange. It must be remembered that the Corning blue filter was transmitting a small percentage of parasitic red rays, but results obtained in the third series indicate that they alone are not responsible for the prolific growth under this screen.

The light intensity and energy transmitted by the control (T and E =71%) were too high for good growth, the amounts transmitted by all the reducing non-selective filters being much more favourable. Under the selective filters the cultures grew well, for although the light intensity was in some cases high, e.g. orange and red, the relative energy in these regions is considerably less than for white light (Table II).

Third Series. Winter and Early Spring. January 2nd–April 14th, 1930.

The cultures were set up in the same manner as before, the only difference being in the use of additional filters, viz. the heat-absorbing

screen which was placed over the Corning blue, and the extra filters of Schott blue, and Chance blue (Nos. 11, 12, and 13, Table II).

Attention is here drawn to the fact that the transmission curves for 11

Fig. 5.

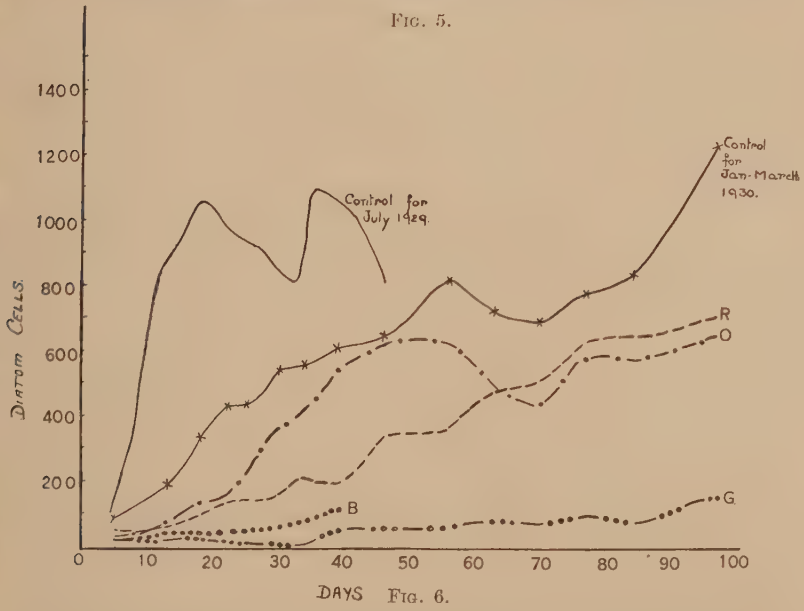

Fig. 6.

Figs. 5 & 6.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium* when grown under screens reducing the light intensity selectively, Fig. 6, lower portion, and non-selectively Fig. 5, upper portion, for a period of 97 days during January-March, 1930. The curve showing growth under the control screen during June and July, 1929, is also shown.

and 12 were obtained from data supplied by the makers in January, 1930, and it has not been possible as yet to verify these figures, but the absence of any appreciable amount of red has been verified by visual spectroscopic examination.

The average number of hours daily sunshine in January was 1 hour 10 minutes, in February 3 hours 18 minutes, and in March 3 hours 50 minutes. Table VIII and Figures 5, 6, and 7 are records of growth for this period.

Conclusions from Third Series.

1. The results suggest that the total light intensity received is a factor of great importance. Excepting the Schott blue filter, the diatoms under the ordinary glass screen show the best growth, whilst the cultures under

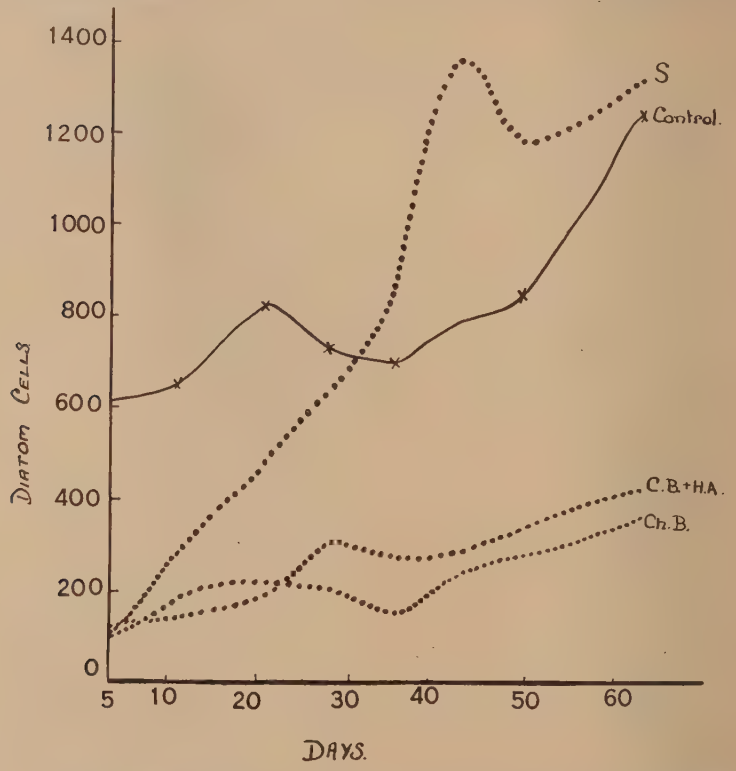

Fig. 7.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium* when grown under the influence of blue light for a period of 63 days during February and March, 1930. The curve showing growth under the control screen during the same period is also given.

the Wratten filters show poor growth where the intensity is heavily reduced. The figures shown in Table IX would suggest that the relative energy is a limiting factor, the ratio $\frac{\text{Relative Energy}}{\text{No. of diatoms}}$ being a constant in the experiments and having a value of 24·5.

TABLE VIII.

To Show the Growth of the sub-cultures of *Nitzschia closterium* grown under Selective and Non-selective Filters from January 2nd–April 9th, 1930, and from February 5th–April 9th, 1930.

Number for diatoms per unit of volume.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of days.	°C.	T=$\frac{1}{2}$	T=$\frac{1}{4}$	T=$\frac{1}{8}$	T=$\frac{1}{16}$	T=$\frac{1}{32}$	Control Glass.	Corning Red.	Corning Orange.	Corning Green.	Corning Blue.	Corning Blue + Heat Ab.	Schott and Gen Blue.	Chance Blue.	No. of days.
—	—	—	—	—	—	—	—	—	—	—	—				
5	13·1	48	36	32	28	28	88	48	48	32	20				
13	13·2	88	52	32	20	17	188	60	72	20	45				
18	13·1	172	84	56	20	20	332	96	136	26	40				
22	11·8	280	108	64	20	20	432	136	160	16	44				
25	10·6	300	200	96	20	8	432	144	224	9	50				
30	10·1	256	220	98	16	8	544	168	368	8	60				
34	12·6	220	224	152	20	12	560	212	428	8	88				
39	9·9	232	236	216	28	12	616	200	544	57	116	116*	108*	104*	5*
46	10·6	320	272	256	60	40	650	340	624	60	—	140	281	184	12
56	13·3	318	272	260	62	40	825	368	630	60	—	192	484	216	22
63	13·2	250	212	240	64	45	728	488	500	88	—	302	640	200	29
70	14·2	200	200	170	66	40	696	516	436	82	—	276	860	150	36
77	11·2	259	200	200	70	43	788	632	576	100	—	280	1360	232	43
84	12·9	266	240	232	76	40	944	652	584	88	—	332	1180	280	50
97	14·1	408	396	456	156	40	1244	716	656	168	—	420	1316	360	63

* Columns 13–16 give the figures for the cultures started February 4th, 1930.

TABLE IX

To Show that the Ratio of the Relative Energy to the Number of Diatoms tends to be a Constant when the Relative Energy transmitted is Low.

Screen Used.	T=$\frac{1}{32}$	T=$\frac{1}{16}$	Corning Green.	T=$\frac{1}{8}$
Percentage Light Transmitted . . .	1·7	3·3	13·5	9·5
Percentage Relative Energy	1·7	3·3	3·3	9·5
No. of Diatoms at the end of 84 days . .	40	76	88	233
$\frac{\text{Relative Energy}}{\text{No. of Diatoms}}$ . .	23·5	23·1	26·3	25·2
Average Ratio . .	24·5			

2. The large growth under the Schott blue filter (T=50·3 and E=14·0) is difficult to account for unless the screen transmits almost the optimum light intensity and energy. This view would explain the relatively poor growths under the Chance blue, and combined Corning and heat-absorbing screens where T=36 and E=10 for the Chance and T=16·7 and E=4·7 for the combination. Under the red filter (T=67, E=30), and the orange (T=73, E=34), the rates of growth were very similar to each other, that under the orange being slightly better than that under the red for the greater part of the third series. Unlike the summer results, the growths under these two filters were poorer than that under the ordinary glass screen. Under the green filter (T=13·5, E=3·3) growth was poor, and suggested the importance of the relative energy transmitted when the light intensity was low (Table IX).

3. The difference in colour exhibited by some of the cultures has already been referred to. When grown in ordinary culture flasks the growths are yellowish brown. Under the coloured screens they vary from a rich dark brown shade to decided greenish tints. Under the reduced light of the non-selective Wratten filters the cultures remained yellowish brown as under the control. These colours are not due to differences in the numbers of diatoms present, for counts made on April 9th gave 1244 per unit of volume under the glass filter and 1316 under the Schott blue, yet the culture under the latter was the rich brown colour, whilst under the ordinary glass the culture was yellowish brown. Under the red and yellow filters the cultures always assumed a decided yellow-green shade, whilst under the blue and green they were decidedly dark brown, i.e. the cultures tended to assume colours complementary to those in which they were grown. The dark brown colour of the cultures under the blue and

green filters is probably an adaptation which helps to compensate the deficiency of red and yellow rays by furnishing an added power to absorb the rays available. This is of interest when it is considered that marine diatoms are frequently abundant between depths of 5–15 metres in the sea, being seldom found very near the surface because of the harmful effect of high light intensity. It is known that at a depth of 5 metres most of the red rays are absorbed, whilst at 15 metres the light that penetrates is reduced to about 10–20% of the total intensity and is deprived of all the red, practically all the yellow, and consists of green and blue light only.

4. The cultures showed the same periodic decrease in numbers of diatoms similar to those of the summer series. Drops of solution from the cultures gave pH values from pH 8·8–9·2, showing that the solutions in which the diatoms grew were strongly alkaline. Whilst silica is highly resistant to acids it yields to alkalines, and it is suggested that the falling off in numbers is due to the fact that the thin shells of the dead frustules are dissolved in the culture solutions. This view is upheld by the results of the following experiments. A certain volume of culture solution containing a known number of diatoms per mm.^{3} was gently heated and kept just below boiling point for some time. The solution was then made up to the original volume with water and counts made for the number of individuals present in the usual way. Nearly all the diatoms had disappeared, suggesting that all but the most resistant frustules had dissolved, the boiling being a quick method of showing what would be a slow process in the culture solutions.

GENERAL CONCLUSIONS.

Three series of cultures of the marine diatom *Nitzschia closterium* were grown under selective and non-selective filters, the transmissions of which were known between definite limits of wave length.

From results of observations on the growth of the cultures it would appear :—

1. That the amount of energy transmitted is of greater importance than the precise wave lengths between which the energy so transmitted lies. When the amount of radiation is small then growth tends to be proportional to the relative energy, but when the energy received is too intense then the effect is harmful to the cultures.

2. At all times the cultures tend to show chromatic adaptation when grown under selective filters, assuming colours which are complementary to those in which they are growing, e.g. a dark rich brown shade under the green and blue screens and a decided green colour under the red and yellow.

Since these experiments were completed, Klugh (1930) has published a further report on the effects of light of different wave lengths, but of equal intensities, on the photosynthetic rates for certain green and red algæ. His results would appear to confirm the conception of the complementary nature of the brown colour.

3. The cultures are subject to periodic decrease in numbers, which is probably due to the thin siliceous shells of the diatoms being dissolved in the culture solutions which have become highly alkaline.

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Studies on *Ligia oceanica*. I. A. Habitat and Effect of Change of Environment on Respiration. B. Observations on Moulting and Breeding.

By
Aubrey G. Nicholls, B.Sc.

With 2 Figures in the Text.

CONTENTS.

PAGE

INTRODUCTION 655

A. HABITAT AND EFFECT OF CHANGE OF ENVIRONMENT ON RESPIRATION

1. Historical 656
2. Habitat 656
3. Immersion Experiments 657
4. Effect of Change of Temperature on Respiration 659

B. OBSERVATIONS ON MOULTING AND BREEDING

1. Historical 663
2. Rate of Growth and Breeding 665
3. Viability of Unfertilised Ova 670

SUMMARY 671

REFERENCES 673

INTRODUCTION.

THIS paper is the outcome of work done at the Laboratory of the Marine Biological Association at Plymouth during the period October, 1929, to January, 1931, while holding a studentship under the Council for Scientific and Industrial Research of Australia.

It gives me pleasure at the outset to express my thanks to the Director and members of the staff at Plymouth for their interest and help in my work, also to the many visitors to the Laboratory who have given much assistance. In particular would I like to thank Dr. C. M. Yonge, without whose help part of this work would never have been attempted.

During the course of sixteen months at Plymouth several aspects of the biology of Ligia were touched on and it is my intention to deal with the subject under three headings. The first, "Habitat and Effect of Change of Environment on Respiration," will include a general description of the nature of the habitat of this animal with reference to its food, reaction to light, animals which prey upon it, and an account of some experiments upon its respiration. These latter were performed during the course of a short visit to the Marine Station at Millport, Scotland, and much of th

success of the results is due to the interest displayed and assistance given by Miss S. M. Marshall and Mr. A. P. Orr. I have also to thank Mr. Elmhirst, the Superintendent, for permitting me to occupy a table at the Station during that period.

The second heading, "Observations on Moulting and Breeding," will cover work which occupied sixteen months during which the animals were watched in the Laboratory and notes taken on their moulting and breeding, with reference to temperature, etc. It will also include experiments to discover the viability of the unfertilised ova. In Part II will be described the structure of the foregut, and the paper will be concluded with a description of the processes of feeding, digestion, and absorption.

A. HABITAT AND EFFECT OF CHANGE OF ENVIRONMENT ON RESPIRATION.

Historical.

Though many workers, particularly in the last decade of the nineteenth century, have been interested in the lower Crustacea, attention was mainly devoted to the Amphipods or, where Isopods were investigated, the terrestrial members of that group received most attention. On the whole Ligia has come in for very little notice, though occupying a very interesting position amongst the Crustacea by virtue of its structure and habitat. Among the more recent workers, Tait (1917) has carried out experiments on immersion, moulting, limb-flexure, and colour change in Ligia; Stewart (1913) has also published the results of experiments on immersion in salt and fresh water. An excellent description of the general morphology of Ligia is provided by Hewitt (1907).

Habitat.

The distribution of Ligia is very wide, covering practically the whole of the north coast of Europe; it has also been recorded from Morocco and America. The natural habitat consists of crevices in rocks just above high-water mark, or where the beach is sandy they will be found under stones and rocks at a similar level. When occupying such an area very large specimens are rare, though females with brood pouches have been found frequently. The largest animals occur on rocky areas, and a particularly favourable spot is to be found along the quays at Plymouth where the mortar has been washed out from between the rocks composing the walls, forming ideal crevices in which to hide.

Ligia is a nocturnal animal, never appearing during the day unless disturbed, and for that reason always difficult to obtain in any quantity by daylight. At night, during low tide, they emerge in large numbers and descend to the lower levels of the walls on which is growing a thick layer

of *Fucus vesiculosus* and other algæ. Here they feed, making the most of the time while the tide is out, and while the seaweed forms the main article of diet, nothing edible comes amiss, particularly if it be of the nature of animal offal. One specimen collected from the region above that affected by the sea-water and apparently feeding on moss was killed and the contents of the gut examined under a microscope. Amongst the material therein contained, moss capsules and part of a syncytium of Vaucheria were easily identified. Evidently this animal is not confined to marine vegetation.

When feeding in this manner, with the aid of a torch specimens can be collected in large numbers by simply walking along the base of the wall and picking the animals off the seaweed. They are quickly disturbed by the light and will retreat to the crevices if not captured quickly. The light of a bright moon is sufficient to prevent them from emerging. How they manage to feed on bright moonlight nights I am at a loss to explain, but experience proved time and again that it was useless to expect to obtain more than a dozen specimens from a wall which on dark nights provided 1000 specimens in just over two hours. Doubtless if they emerged during the day they would be preyed upon by sea-gulls and other birds, and as it is, when feeding at night, they are liable to fall a prey to the common shore crab, *Carcinus mœnas*. I have observed on several occasions an unfortunate Ligia caught, by a sudden movement of these usually slow moving animals, in the chelæ of a crab.

Only very young individuals are found in the open during the daytime and then not very abundantly. The young seem to have much less aversion to light than the mature animals, this being noticed in those bred in the laboratory as well as in nature.

Immersion Experiments.

Though preferring a moist locality above high water, Ligia can withstand immersion for a considerable time in salt water. Stewart (1913) immersed a number of Ligia in fresh pond water and others in sea-water, but apparently took no measures to aerate the water with the natural result that all died within 48 hours, those in fresh water living longer than those in salt. These experiments were repeated, the water being changed continually with the result that they survived nine days, death being ascribed rather to lack of food than to the ill-effects of immersion. Tait (1917a) showed that specimens immersed in sea-water survived for more than 83 days (2 specimens); those immersed in half sea-water lived for, at the most, 42 days; those in quarter sea-water only 15 days, while others immersed in distilled water lived only a matter of hours. Care was taken in every case to aerate the water. He showed, moreover, that during that period of immersion those living in clean sea-water fasted, and moulted normally.

During the course of my investigations I carried out similar experiments with animals of varying ages, employing the method in use by Sexton and Clark of gradually changing the salinity of the water by adding a constant amount of fresh water daily, having first removed a similar quantity. The volume was kept constant by the addition of distilled water to counteract evaporation. The water was aerated by passing a fine stream of air through it and a small piece of Fucus was placed in each jar for food and as an object of attachment for the Ligia. This, though not important, is useful, as otherwise the animals will wander round and round the bottom of the jar, periodically swimming vigorously about in an apparent attempt to escape. If only a stone or shell be placed at the bottom Ligia will soon settle down upon it.

Two jars, K1, E1, were employed containing each about 300 c.c. sea-water, and four, K2, E2, E3, E4, containing each 250 c.c. Into each jar were placed three small Ligia on 2nd December, 1929. In the two jars, K1, K2, used as controls, the level of the water was maintained by the addition of distilled water as required, and of the remaining four 5 c.c. of water from E1, 10 c.c. from E2 and E3, and 25 c.c. from E4 were removed daily, a similar quantity of fresh (tap) water being substituted. Moulting was observed to occur in these young specimens.

Those undergoing the quickest dilution died first, surviving only five days, while those in E2 and E3 were dead by 14th December, having survived less than 12 days. There were animals still alive in K2 and E1 at the end of the month.

An interesting record was that of an adult animal which at some time had fallen into a large jar of sea-water and become quite at home. This jar was taken as a whole and placed under the system of aeration and dilution (E5), 25 c.c. being removed daily from a total volume of about 2 litres. This started on 10th December.

It was noticed that the rate of beating of the pleopods appeared to be considerably faster than when placed in sea-water, accordingly the time for 100 beats was measured with a stop-watch, as follows :—

On 24th	December	time for 100 beats	60·8	secs.
25th	,,	,,	60·0	,,
29th	,,	,,	56·9	,,
5th	January	,,	53·5	,,
13th	,,	,,	50·0	,,

showing a steady increase in rate of beating with further dilution of the sea-water. This was compared with the rate of beating of another specimen which had been in sea-water for some time and it was found that this animal beat its pleopods very rarely and then only for a short time and very slowly unless disturbed, when it would beat at the rate of 50 beats

in 65·7 secs. On January 13th this specimen, E5—a female—was due to moult but succumbed in the attempt, probably due to asphyxiation during the process of liberating its pleopods.

While at Millport specimens had been collected from beneath stones and small rocks that were moistened by a stream of fresh water running into the sea, and when the tide rose were actually covered by water which would have a very reduced salinity. Water from this pool was kindly tested by Mr. Elmhirst and was shown to have a salinity only slightly above that of normal fresh water.

Effect of Change of Temperature on Respiration.

The following series of experiments carried out at Millport gives some idea of the effect of change of temperature on the animal as a whole, though most strikingly demonstrated by the change in rate of beat of the pleopods. The first shows the effect of sudden change in temperature.

Three specimens were placed in sea-water at ordinary room temperature, and thermos flasks were prepared containing water at definite temperatures. Into the neck of each flask was fitted a test-tube containing sea-water at the same temperature as that in the flask; the tubes were corked to prevent loss of heat, and provided with thermometers. The animals for experiment were then transferred to these tubes with the following results :—

TABLE I.

Effect of Sudden Change in Temperature on Ligia Immersed in Sea-water.

At 4.35 p.m.	Specimen A was placed in sea-water at 25° C.
5.03 p.m.	apparently unharmed.
6.00 p.m.	changed to flask at 35° C.
7.35 p.m.	temperature down to 30° C., animal almost dead, transferred to water at 15° C.
8.35 p.m.	quite recovered.
At 4.35 p.m.	Specimen B was transferred to sea-water at 30° C.
5.03 p.m.	still living, temperature 29° C.
5.53 p.m.	apparently dead, temperature 29° C., transferred to water at 15° C.
7.35 p.m.	had not recovered.
At 4.35 p.m.	Specimen C was transferred to sea-water at 35° C.
4.43 p.m.	unable to flap pleopods, tending to curl up, to all appearances dead, replaced in cold sea-water.
5.23 p.m.	completely recovered.

Thus it will be seen that the greater the rise in temperature the shorter the period of survival and yet though apparently dead, that is when all movement of the pleopods has ceased, if transferred to more normal conditions the animal may recover. Specimen C withstood a temperature of 35° C. for 8 minutes and then recovered, though apparently dead. Specimen B having been subjected to 30° C. for half an hour was unaffected, though a further 50 minutes was sufficient to kill it.

This experiment led naturally to an attempt to discover the lethal temperature for this animal when subjected to a gradual increase in temperature, and a comparison of its survival in air under those conditions with its survival in water.

A large tank was set up filled with water which was stirred mechanically and the temperature controlled by a thermostat. Into this tank was placed a number of small bottles each containing one Ligia, 30 specimens being used in all. Half of these bottles were filled with sea-water and the other half contained only the animals, five adult males, five adult females, and five young specimens being used in each experiment. In order that those immersed in water should not suffer from lack of oxygen the water was changed at regular intervals, care being taken to ensure that the added water was at the appropriate temperature. Each bottle was numbered and those filled with air were fixed into a wire basket and weighted to keep them properly submerged.

The water in the thermostat was raised 1·0° C. every hour and kept constant for that period. The sea-water inside the bottles was changed once every three hours. The time of death of each animal was noted along with the temperature. This information will be found summarised in Table II.

TABLE II.

Showing Temperature at which Death Occurred for Individuals in Air and in Water.

Temp. °C.	28	30	31	32	33	34	35
			IN AIR.				
Males	–	–	–	–	–	2	3
Females	–	–	–	–	1	2	2
Young	–	–	–	–	–	5	–
			IN WATER.				
Males	1	2	–	2	–	–	–
Females	–	–	–	1	2	1	1
Young	–	1	–	1	1	2	–

TABLE III.

Showing Number of Beats per minute with increased Temperature in Males, Females, and Young.

No.	Temperature in degrees Centigrade. 12	15	16	17	19	21	23	25	27	29	31	33	35
							Males.						
1.	93·8	114	121	131	146	164	174	173	204	231	Dead		
2.	90·9	108	115	122	136	159	177	171	200	200	Dead		
3.	90·9	112	121	123	160	174	171	180	213	216	113	Dead	
4.	107	118	121	133	152	170	177	172	201	222	217	Dead	
5.	95·3	116	118	121	155	155	189	172	183	201	Dead		
Mean	95·6	114	119	126	150	164	178	174	200	214	—		
							Females.						
1.	88·2	107	115	131	155	174	197	179	211	224	217	Dead	
2.	79·5	113	119	132	155	173	184	184	227	227	211	Dead	
Mean	83·9	110	117	132	155	174	191	182	219	226	214	—	
							Young.						
1.	109	—	135	154	186	195	200	189	224	240	294	309	Dead
2.	112	—	160	181	206	216	203	217	246	259	300	316	Dead
3.	113	—	156	163	188	201	210	221	250	259	300	306	Dead
Mean	111	—	150	166	193	204	204	209	240	253	298	310	—

The lethal temperature in air lies at about 34° C., though a certain proportion survived that temperature for one hour and withstood for a short time a further rise to 35° C., though none for more than 10 minutes. When immersed in water the adult males seem to be much less able to withstand any increase in temperature above 30° C., while the majority of adult females and young of both sexes succumbed between 32° and 34° C.

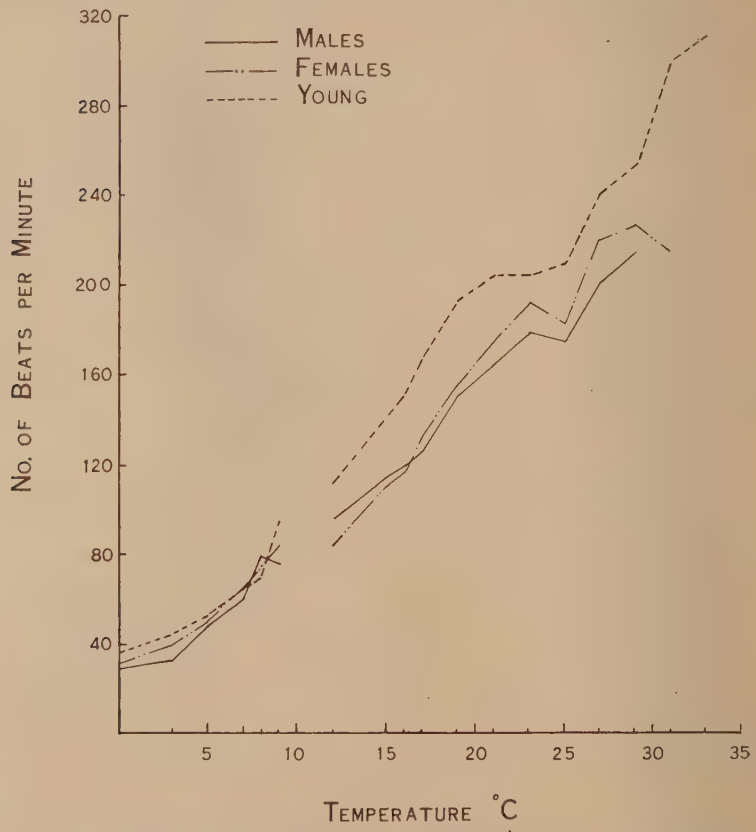

FIG. 1.—Graph showing the change in rate of beating of the pleopods with change in temperature; drawn from the averages of the males, females, and young in Tables III and IV.

Respiration seems to be the limiting factor for immersed animals; the continued beating of the pleopods to obtain sufficient oxygenation of the blood while immersed, and the consequent drain on the energy of the animal would account for the earlier death of immersed animals compared with those in air. It was apparent that as the temperature was increased

the rate of beat of the pleopods also increased. With a view to obtaining data on the increase in rate of beat the above experiment was repeated on a number of animals immersed in water, the time taken for 100 beats being measured with a stop-watch at the end of each hour. In addition to starting at room temperature and gradually increasing it another experiment was run simultaneously in which, by adding ice, the temperature was lowered finally to zero. The results of these experiments, expressed as the number of beats per minute, will be found in Tables III and IV and depicted graphically in Figure 1.

TABLE IV.

Showing Number of Beats per Minute with Decreased Temperature in Males, Females, and Young.

No.	Temperature in degrees Centigrade. 9	8	7	5	3	0
			Males.			
1	75·8	75·8	64·4	56·0	35·3	29·9
2	76·5	82·0	56·2	39·9	30·7	28·7
Mean	76·2	78·9	60·3	48·0	33·0	29·3
			Females.			
1	92·5	82·4	72·1	59·3	42·0	34·3
2	75·8	67·6	59·1	40·2	37·7	28·7
Mean	84·2	75·0	65·6	49·8	39·9	31·5
			Young.			
1	96·8	74·3	69·9	56·8	48·9	36·1
2	92·6	65·2	60·0	49·5	41·6	36·9
Mean	94·7	69·8	65·0	53·2	45·3	36·5

On reference to Figure 1 it will be seen that in effect it is a straight line graph, indicating a constant change in rate of beat as the temperature rises or falls between 5° and 35° C. Between 5° and 0° C., however, there is a tendency for the rate to become constant.

B. OBSERVATIONS ON MOULTING AND BREEDING.

Historical.

In October, 1929, a number of Ligia were brought into the laboratory and kept under observation to watch the process of moulting and in the hope of inducing them to breed. Since they were found under natural conditions feeding on Fucus it was thought that they might survive for some time if placed on moist Fucus in a bowl and covered to prevent evaporation. To this end a number of finger bowls were employed, in each were placed a piece of *Fucus vesiculosus* and a pair of animals to be

observed and they were then covered with glass squares. Each bowl bore a numbered label.

It was found by experience sufficient to wash the bowls and weed in sea-water two or three times a week and to change the weed about once a fortnight during the winter. In summer more frequent changing of the weed was required, with washing on alternate days. Under these conditions the animals lived, fed, moulted, grew, and bred apparently quite normally for several months, some surviving 15 months before being killed, while one is still alive at the time of going to press.

The cast cuticles of each animal, when not eaten, were preserved (70% alc.) and the animals measured as soon as the new cuticle had hardened after the process of moulting. Notes were taken of any changes in colour of the skin or eyes, condition of brood plates and other points of interest. From the information obtained it has been possible to reconstruct a fairly complete history of the life of the animal subsequent to emergence from the brood pouch.

As stated by Hewitt, the body is broadest across the fourth (free) thoracic segment, gradually decreasing in size posteriorly. This statement applies to all normal individuals with the exception of females which have developed mature brood plates, in which case the greatest breadth is in the third free thoracic segment, and by means of this distinction in shape animals with brood pouches can be picked out at a glance quite easily.

The colour of the eyes is normally black, but in a certain proportion, particularly females, a chocolate brown occurs which is most noticeable after each moult, gradually darkening as the new " skin " grows older. In one young specimen found towards the end of 1929 the colour of the eyes was noticed to be very bright red, while the general body colour was very pale, almost white, probably due to lack of development of chromatophores.

The process of moulting has been described by Tait (1917b) and it will suffice here merely to add the results of a few of my observations. With reference to the " chalky whiteness " which appears some time before moulting on the ventral side of the first four free thoracic segments, rough tests of its composition made by me support the statement of Herold (1913) that it is a deposition of calcium carbonate. A gas that will not support combustion is evolved when treated with acid, and the flame test indicates calcium. This deposit appears a number of days before moulting, the period varying with the time of year and the age of the animal, as does also the interval between the posterior moult and the anterior. The longest times are shown in adult males during winter (average laboratory temperature about 14° C.), when the first appearance of the deposit may precede the moult of the posterior portion by six days and there are four full days between the moulting of the posterior and anterior portions ; while the shortest times occur in young specimens in summer, when the

first appearance of the deposit, the posterior moult and the anterior moult follow one another on successive days. The average laboratory temperature in summer was about 18° C. Further it may be worth noting that, though there was little or no tendency for Ligia to eat their cast "skins" during the earlier part of their captivity, towards the end of the period this tendency became more and more marked until it was seldom that one could rescue a complete moult for preservation.

Rate of Growth and Breeding.

In Table V will be found figures showing the total increase in size for a number of animals over different periods. It was noticed that members of both sexes grew until a certain size was attained, after which further moulting showed no increase.

TABLE V.

Showing Total Growth over a Given Period for a number of Animals.

No.	Date of first Measurement.	Size in mm.	Date of last Measurement.	Size in mm.	Total Growth mm.	Time in Months.
			Females.			
1.	25/10/29	20·5 × 9·0	27/5/30	22·5 × 10·0	2·0 × 1·0	7
4.	25/10/29	23·0 × 10·0	4/5/30	24·5 × 11·0	1·5 × 1·0	6¼
5.	12/11/29	24·5 × 10·5	11/8/30	27·5 × 12·0	3·0 × 1·5	9
7.	31/10/29	22·5 × 10·5	4/7/30	25·5 × 11·0	3·0 × 0·5	8¼
8.	31/10/29	23·2 × 11·0	1/7/30	26·5 × 11·5	3·3 × 0·5	8
9.	12/11/29	22·8 × 10·0	3/7/30	27·0 × 12·0	4·2 × 2·0	7¾
10.	22/10/29	22·0 × 10·0	18/2/30	23·8 × 11·0	1·8 × 1·0	4
15.	12/11/29	23·7 × 10·3	4/5/30	26·0 × 11·5	2·3 × 1·2	5¾
18.	12/11/29	22·0 × 10·0	8/5/30	23·8 × 11·5	3·8 × 1·5	6
19.	12/11/29	21·0 × 9·5	23/12/30	25·8 × 11·0	4·8 × 1·5	13½
20.	12/11/29	24·5 × 10·2	9/1/30	26·0 × 11·5	1·5 × 1·3	2
21.	12/11/29	20·0 × 9·0	4/8/30	23·0 × 10·0	3·0 × 1·0	8¾
22.	12/11/29	22·3 × 9·5	18/2/30	24·0 × 10·5	1·7 × 1·0	3¼
23.	12/11/29	22·0 × 9·5	18/6/30	25·0 × 11·0	3·0 × 1·5	7¼
24.	12/11/29	20·0 × 9·0	5/2/30	23·2 × 10·0	3·2 × 1·0	2¾
25.	12/11/29	25·8 × 11·5	11/12/29	27·5 × 12·3	1·7 × 0·8	1
			Males.			
2.	28/10/29	30·0 × 14·5	25/4/30	31·8 × 15·5	1·8 × 1·0	6
6.	28/10/29	29·2 × 13·8	20/12/29	30·3 × 14·0	1·1 × 0·2	1¾
15.	5/11/29	27·0 × 11·5	19/6/30	29·5 × 14·0	1·5 × 2·5	7½
19.	12/11/29	26·7 × 12·4	8/9/30	30·0 × 15·5	3·3 × 3·1	10
20.	12/11/29	25·0 × 13·0	24/3/30	27·4 × 13·8	2·4 × 0·8	3½
21.	12/11/29	23·0 × 10·2	6/6/30	29·0 × 14·5	6·0 × 4·3	6¾
22.	12/11/29	23·0 × 10·5	4/4/30	28·6 × 14·8	5·6 × 4·3	4¾
23.	12/11/29	25·0 × 11·0	8/11/30	30·0 × 14·0	5·0 × 3·0	12
24.	12/11/29	22·7 × 10·3	26/12/29	24·0 × 12·0	1·3 × 1·7	1½
27.	21/11/29	30·0 × 14·7	30/3/30	31·5 × 16·5	1·5 × 1·8	4¼
37.	23/12/29	31·0 × 15·7	24/4/30	32·2 × 17·0	1·2 × 1·3	4
			Young.			
12.	22/10/29	3·0 × 1·3	18/3/30	7·5 × 3·3	4·5 × 2·0	5
14.	22/10/29	3·0 × 1·3	23/12/30	20·5 × 8·5	17·5 × 7·2	14
26.	21/11/29	6·1 × 2·6	23/12/30	20·0 × 8·75	13·9 × 6·15	13

Two young (3·0×1·3) liberated from the brood pouch on 22nd October, 1929, were kept alive in the laboratory. These both moulted within a fortnight and again at the end of a second fortnight, this being continued with gradually increasing intervals until April, 1930, by which time the interval between moults was just over a month. Growth was not the same in both cases, No. 12 moulting a few days before No. 14 and showing a greater increase with each moult. By the end of January, 1930, moreover, after the sixth moult the larger specimen was discovered to be a male, the appendages appearing in the " skin " of the 7th moult. No. 14 at this time showed no signs of secondary sexual characters and was assumed, therefore, to be a female, the brood plates being expected to appear at a later stage. At this time, March 16th–18th, their respective sizes were, male : 9·2×4·0 mm. and female : 7·5×3·3 mm., and from now until the beginning of September the interval between the moults decreased, averaging about 2½ weeks.

The size attained by April was such that it was found impracticable to measure the animals accurately. They were still too small to be held in the fingers as larger animals were treated and were too strong to be held on the measuring slide with a brush, the method employed on them up to date. Thus no measurements were taken through April, May, June, and July. During the latter month No. 14 escaped, a most unfortunate loss. It was not until the moult of July 2nd that the brood plates appeared in this animal and it was noticeable that after this moult it was larger than the male, having also decreased the interval between moults relative to the male, so that now No. 14 was moulting a week before No. 12, a complete reversal of the previous state of affairs. It was at this most interesting point that the specimen was lost.

The male, No. 12, continued to grow, and by the end of July had attained to 15·0×6·5 mm. Five successive moults brought it to 20·5×8·5 mm. at the end of December and after moulting again in January, 1931, was fixed at the beginning of February, having survived 14½ months. There is apparently only a very slight variation in the rate of growth in summer and winter, the specimen holding very closely to an increase of 1·3×0·5 mm. at each moult, which was the average for the whole period. The summarised histories of these two will be found in Table VI.

To return to the female. Had not the specimen been lost it is safe to assume that it would have been at least as large as the male in December (in view of its sudden increase in size relative to the male in July), by which time it would have reached maturity and would have been able to produce a brood ; in support of this we have the evidence of three females (No. 43) who all produced broods on attaining a size of 22·0×9·5 or 10·0 mm. and also the record of one female measuring only 19·0×8·5 mm. which was found in the natural state with a brood. Thus we see that within 16

months of birth, probably earlier, a female may produce her first brood of young. Further, we see that by June, when the second brood might be expected, the animal has attained a size of 24·5 × 10·0 mm. (No. 21) and from the history of No. 16 we see that this animal after having a period of rest through the autumn might start breeding again in January with a fourth brood in May and a fifth in August, most probably dying after liberating that brood in November.

TABLE VI.

	Number 12.			Number 14.		
	Date.	Size.	Int. bet. Moults days.	Date.	Size.	Int. bet. Moults days.
Birth	22/10/29	3·0 × 1·3	—	22/10/29	3·0 × 1·3	—
1st Moult	—	—	(15)	—	—	(15)
2nd „	22/11/29	4·3 × 1·7	(16)	22/11/29	3·8 × 1·7	(16)
3rd „	8/12/29	4·6 × 2·0	16	7/12/29	4·5 × 1·9	15
4th „	24/12/29	5·3 × 2·5	16	24/12/29	5·0 × 2·1	17
5th „	10/1/30	5·9 × 2·5	17	11/1/30	5·6 × 2·5	18
6th „	*27/1/30	7·0 × 3·2	17	29/1/30	6·0 × 2·5	18
7th „	21/2/30	8·0 × 3·5	25	23/2/30	6·5 × 3·1	25
8th „	16/3/30	9·2 × 4·0	23	18/3/30	7·5 × 3·3	23
9th „	10/4/30	—	25	11/4/30	—	24
10th „	11/5/30	—	31	12/5/30	—	31
11th „	6/6/30	—	26	4/6/30	—	23
12th „	23/6/30	—	17	17/6/30	—	13
13th „	9/7/30	—	16	*2/7/30	—	15
14th „	26/7/30	15·0 × 6·5	17		Lost.	
15th „	15/8/30	16·5 × 6·8	20	*N.B.* Female at this stage larger than male.		
16th „	2/9/30	18·4 × 7·0	18			
17th „	28/9/30	—	26			
18th „	30/10/30	—	32			
19th „	9/12/30	20·5 × 8·5	40			
20th „	30/1/31 Fixed.	—	52			

* Indicates appearance of secondary sexual characters.

This hypothetical case has been constructed from data obtained from the records of actual specimens, only the results from animals of comparable size being taken into consideration. The records of these animals referred to in the text will be found in Table VII.

TABLE VII.

Extracts from Histories of Nos. 21, 24, and 16.

No.	Date of Moult.	Size.	Date of Moult.	Size.	Date of Moult.	Size.
21.	25/12/29	23·0 × 9·1	†7/2/30	23·0 × 10·1	3/5/30	24·0 × 10·2
	†4/6/30	24·5 × 10·0				
24.	25/12/29	23·5 × 9·6	†5/2/30	23·2 × 10·0		
16.	†22/5/30	25·0 × 11·0	22/7/30	26·0 × 11·0	†19/8/30	

† Indicates mature moult with brood.

Thus it will be seen that the expected life of a female is just over three years, the first being one of active growth, maturity being reached early in the second with the production of perhaps two broods, while the third year might see the release of three broods, culminating in the natural death of the animal in winter. The average number of young produced in each brood is about 80, a few cases of over 100 having been found, and sometimes as few as 40, varying with the age of the animal.

In Table VIII will be found the dates of spawning of the animals kept in the laboratory. Through the winter the average period taken by the eggs to develop and be liberated from the date of spawning was about 90 days, the young embryos becoming pigmented at about 40 days ; as the summer approached this period was gradually reduced until a period of 40 days only was required for the whole process during mid-summer, No. 21 spawning on 5th June, pigmentation starting 27 days later, and the brood being liberated on 15th July.

TABLE VIII.

Showing Dates of Spawning and Months of Year in which Females were found with Brood Pouches.

No.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1.	27/30	—	—	—	30/30	—	—					
2.		18/30	—	—								
4.	—	—	—	—	6/30	—	—					24/29
5.	—	—	—	—	10/30	—	—					31/29
6.		28/30	—	—	—							
7.	30/30	—	—	—								
8.			21/30	—	—			←	←	←		
9.			26/30	—	—							
10.		20/30	—					←	←	←		
15.	—	—	—		6/30							29/29
								←	←	←		
16.	20/30	—	—		25/30	—	—	21/30	—	—		
17.	—	—		10/30	—	—						5/29
18.		14/30	—	—								
19.	14/30	3/31	—	—		19/30	—	—				
20.	10/30	—	—									
21.		9/30	—	—		5/30	—					
22.		20/30	—	—								
23.			7/30	—	—	19/30	—					
24.		7/30	—	—								
25.	—	—										13/29
27.	—	—	—	15/30	—	—						15/29
29.	13/30	—	—									
30.	—	—	—	30/30	—	—						27/29
31.	—	—										10/29
32.	15/30	—	—									
33.	9/30	—	—									
34.	7/30	—	—									
35.	6/30	—	—			(7)/30	—					
36.	4/30	—	—		6/30	—	—					
37.	12/30	—	—									
39.	13/30	—	—									
40.	12/30	—	—		17/30	—	—					
41.	3/30	—	—		6/30	—	—					

In Table VIII the figures refer to the day and year, being placed in the appropriate month column, the date being that of spawning. The dashes following each date indicate the months during which the brood was developed and liberated. The arrows (reversed) show that the specimen when collected possessed mature brood plates but that the brood had been liberated, e.g. No. 8 collected in October, found to have mature brood plates but young liberated, must have spawned in August and have had young in pouch through September into October.

From this table it will be seen that Ligia may be found with a brood during any month of the year, though the spring appears to be the main

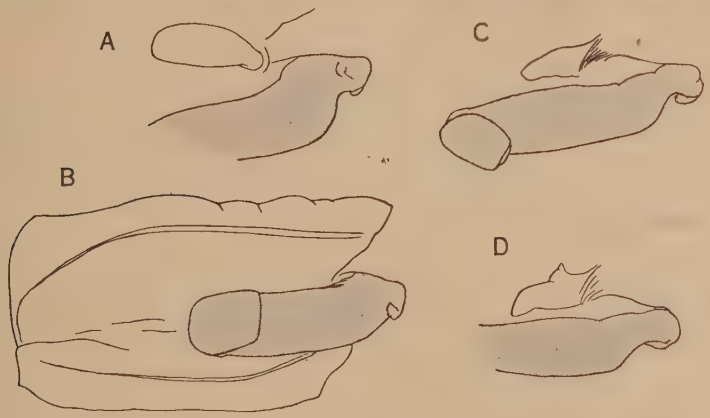

FIG. 2.—A, B, and C illustrate the form of the brood plates in three successive moults (drawn from No. 19). A. Normal condition. B. Mature condition. C. Reduced condition, always followed by a return to the normal. D. Reduced, with anterior projection; a modified form of C which occurs occasionally (drawn from No. 41). All drawn to the same scale.

breeding season, if that term can be applied to any part of the year in particular.

In the normal course of events about 10 days after the brood has been liberated the female moults, the mature brood plates (Fig. 2, B) being replaced with small ones, even smaller than those of the "normal" state (Fig. 2, A) in which they are rounded projections from the ventral sternites of the first five free thoracic segments of the body. Instead of being rounded, these "reduced" plates are pointed (Fig. 2, C). This condition is invariably followed by a return to the normal condition at the next moult. On a few occasions, without any relationship to temperature, season or any other factor apparently, a variant of the reduced condition appeared, the plates being pointed but each bearing a small projection on the anterior edge of the plate (Fig. 2, D). This state was followed by a return to the normal.

Viability of Unfertilised Ova.

The following is an abstract from the history of the pair of animals, No. 23.

March	1st.	Male carrying female ; latter about to moult. Male removed, female left to spawn alone.
	2nd.	Female moulted posterior.
	4th.	Female moulted anterior.
	7th.	Spawned.
(About May	14th.	Young should have been liberated.)
	24th.	Female moulted posterior.
	26th.	Female moulted anterior.
	29th.	Male replaced.

Assuming that during the period in which the male was carrying the female spermatozoa were transferred to her, then it is obvious that these must have been cast off with the moult of 2nd–4th March, since the eggs did not develop, and thus, in order that the ova should be fertilised, copulation must occur subsequent to the moulting of the posterior portion at least. When the moult of the male coincides with that of the female, as happened in several instances, the male would be unable to effect the transference of sperm at the times of moulting each portion and thus it would be advantageous that the ova should remain fertilisable until the male had completed the moult. (In a state of nature it is not likely that the animals pair or that any one female is restricted to any one male and, therefore, the state of affairs described above would be purely due to laboratory conditions.) Nevertheless, it may be seen from the following extracts from the histories of two pairs of animals that the ova were fertilised despite the overlap of the moulting of male and spawning of female.

No. 32.	Jan. 13th.	Female moulted anterior.
	14th.	Male moulted posterior.
	15th.	Female spawned.
	17th.	Male moulted anterior.
	Eggs fertile.	
No. 36.	Jan. 2nd.	Female moulted anterior.
	3rd.	Male moulted posterior.
	4th.	Female spawned.
	6th.	Male moulted anterior.
	Eggs fertile.	

In both these cases the interval between the posterior and anterior moult of the male, during which the female spawned, was sufficient for

the male to recover from the posterior moult and to fertilise the ova before proceeding to slough the anterior portion, but if for any reason copulation could not occur until the male had completed the moulting of the anterior, then a period of three days must elapse subsequent to the appearance of the eggs in the brood pouch before they could be fertilised. That they can survive this period is demonstrated by the following cases where the animals were separated for varying periods, the period of separation being the interval between the appearance of the eggs in the brood pouch and the reuniting of the male and female in the same bowl.

No. 16.	24 hours	separation.	Eggs infertile.
21.	40 hours	,,	Eggs fertile.
19.	3 days	,,	Eggs fertile.
6.	14 days	,,	Eggs infertile.
41.	35 days	,,	Eggs infertile.
23.	83 days	,,	Eggs infertile.

No explanation for the infertility of No. 16 can be put forward other than the possibility of sterility in the male due to age ; the male was full grown and died an apparently natural death in July, about six weeks after the date of spawning.

It is certain, however, that copulation must occur after the female has moulted the posterior portion and may be delayed until certainly three days after the eggs have appeared in the brood pouch. This indicates that the sperm are liberated into the cavity of the brood pouch rather than being passed into the oviducts. Copulation was observed in one case (No. 5) after the female had moulted the posterior portion and before moulting the anterior, in which case if the sperm were not passed into the oviducts they must have been retained beneath the brood plates of the fifth free thoracic segment. Possibly, of course, copulation was repeated after the completion of the anterior moult, certainly the eggs were fertilised.

SUMMARY.

1. Ligia is a nocturnal animal inhabiting crevices in rocks above high-tide level. It is omnivorous though Fucus spp. form the main article of diet.

2. Immersion experiments were carried out in which the animals were subjected to a gradual transition from salt to fresh water. None survived this treatment for more than one month. It was noticed that the reduction in salinity was accompanied by an increase in the rate of beat of the pleopods.

3. Animals were subjected to sudden and gradual changes in temperature. One specimen survived a change from water at 15° C. to water at

30° C. for 28 minutes, but was dead after 78 minutes ; a second, changed from water at 15° C. to water at 35° C., was to all appearances dead after 8 minutes, but recovered after being replaced in water at the original temperature.

4. The lethal temperature for Ligia, when subjected to gradual rise, was determined for males, females, and young in water and in air. Those in water died first, the males by the time 32° C. was reached, the others succumbing between 32° and 34° C. The lethal temperature in air lies about 34° C., a few surviving 35° for a short time.

5. The change in rate of beating of pleopods with change in temperature was determined for immersed males, females and young. The temperature of the water was raised from room temperature to the lethal temperature and lowered from room temperature to zero, the rate of beating being measured at regular intervals. The change in rate of beating is proportional to the change in temperature between 5° and 35° C. Below 5° C. the rate of beating tends to become constant.

6. A number of Ligia were kept in the laboratory for a long time and moulting and breeding observed. The greatest interval between moults occurs in full grown males in winter, the shortest in young specimens during the summer.

7. The growth rate was observed in a young specimen to average 1·3 mm. increase in length and 0·5 mm. in width per month. The growth of a number of adults is tabulated.

8. A hypothetical reconstruction of the life of a female has been made from the histories of several specimens. It is estimated that the length of life of Ligia is 3 years, with a probable production of at least 5 broods of young. The average number of young per brood is 80.

9. The time taken for the development of the young varies from 40 days in summer to 90 in winter.

10. The greatest number of animals with brood pouches is found in spring, but breeding occurs throughout the whole year.

11. In the moult following the liberation of the young the brood plates assume a shape (the reduced condition) distinct from that at any other time. This is followed by a return to the normal condition at the next moult. The reduced form is subject to variation in individuals.

12. Experiments indicate that copulation must occur subsequent to the appearance of the mature brood plates in the female. The ova are spawned about two days after the completion of the moult and, if unfertilised, remain viable for at least 3 days subsequent to their appearance in the brood pouch.

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Studies on *Ligia oceanica.* Part II. The Processes of Feeding, Digestion and Absorption, with a Description of the Structure of the Foregut.

By

Aubrey G. Nicholls, B.Sc.

With 1 Plate and 12 Figures in the Text.

CONTENTS.

PAGE

1. Food and Feeding 675
2. Structure of the Gut
   (*a*) Introductory 676
   (*b*) The Foregut
      (i) The External Form and Musculature 676
      (ii) Internal Structure 677
   (*c*) The Hepato-pancreas 683
   (*d*) The Intestine 683
   (*e*) The Rectum 686
3. Physiology of the Gut 686
4. Comparison with that of the Decapods 689
5. Digestion
   (*a*) The pH of the Gut 689
   (*b*) The Digestive Enzymes 691
   (*c*) The Sucroclastic Enzymes 692
   (*d*) The Proteoclastic Enzymes 696
6. Absorption
   (*a*) Introductory 699
   (*b*) Methods and Results 700
7. Summary 701
8. References 703

1. FOOD AND FEEDING.

The normal food of Ligia is *Fucus vesiculosus*, though other species of Fucus and even Laminaria are eaten. Fine epiphytic algæ which form furry growths upon the surface of the larger seaweeds when growing in places sheltered from the full force of the waves are also eaten extensively. Ligia is not, however, entirely vegetarian in its diet, having definite tendencies towards scavenging and even cannibalism, as observed by other workers who have kept this animal in captivity for any length of time.

The method of feeding is best described as "browsing." The animal clings to its food and cuts off very small portions with its mandibles, passing them through to the œsophagus, whence they arrive at the foregut.

Owing to the animal's dislike of bright light it feeds at night and, since its food is found on the Fucus zone, half-tide is required before it can feed; thus there is only a limited period during every twenty-four hours during which feeding can occur. It is, therefore, necessary that the food taken in should be dealt with as quickly as possible and the animal can be observed feeding voraciously, passing a continuous stream through its alimentary canal, the fæces containing nearly as much undigested as indigestible food.

2. STRUCTURE OF THE GUT.

(*a*) Introductory.

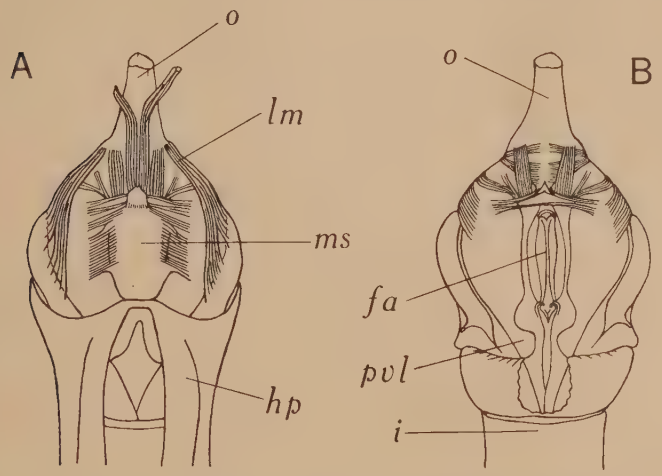
One of the earliest workers on Crustacea to refer to the armature of the foregut, or stomach as it was then called, was Sars (1867). Subsequently Huet (1883), Rosenstadt (1888), Ide (1892), and Schonichen (1898) studied the alimentary canal and its armature in practically every group of Crustacea. Ide concluded that the stomach of the Edriophthalmate orders (Tanaidacea, Isopoda and Amphipoda) was built on the same structural plan as that of the Decapoda, whilst Gelderd (1906) found homologies between the Schizopoda and the Decapoda. Hewitt (1907) described the structure of the gut of Ligia, but ascribed to some parts of the foregut names which are somewhat misleading. Rehorst (1914), in his paper "Der Filtermagen von *Asellus aquaticus*," gives an admirable résumé of the conclusions of previous authors on this subject, as well as giving a detailed description of the foregut of Asellus and assigning functions to its various parts. Barnard (1924) deals with the general shape of the foregut in Isopods, but does not go into detail of structure. Finally, we have detailed accounts of the foregut in Astacus by Jordan (1904), in Homarus by Williams (1907), and in Nephrops by Yonge (1924).

(*b*) The Foregut.

(i) *The External Form and Musculature.*

The main part of the foregut lies in the cephalic and first free thoracic segments, supported by the sternal alæ of the endophragmal skeleton (see Jackson [1926], p. 899, and Lloyd [1908]). Viewed dorsally it is rounded in front, tapering slightly posteriorly, its length being twice its greatest width. The intestine envelops about two-thirds of its posterior portion, only the anterior portion being closed in on all sides, the hinder region consisting of plates which project freely into the intestine.

A general idea of the musculature will be obtained from Text-Figure 1. On the ventral surface are muscles attached to the ventro-lateral walls of the gut and running towards the middle line, where they meet and form a sheath (*m.s.*) to the structure lying immediately above. To the anterior end of the sheath is attached a pair of muscles which can be traced forwards and upwards, separating round the œsophagus and finding their



TEXT-FIG. 1.—A, Foregut and hepato-pancreas from ventral surface showing musculature of foregut. B, same with hepato-pancreas, lateral muscles, muscle sheath, and portion of intestine removed showing some of internal structure of foregut through the ventral wall.

f.a., filter apparatus; *h.p.*, hepato-pancreas; *i.*, intestine; *l.m.*, lateral muscle; *m.s.*, muscle sheath; *o.*, œsophagus; *p.v.l.*, posterior ventral lamella.

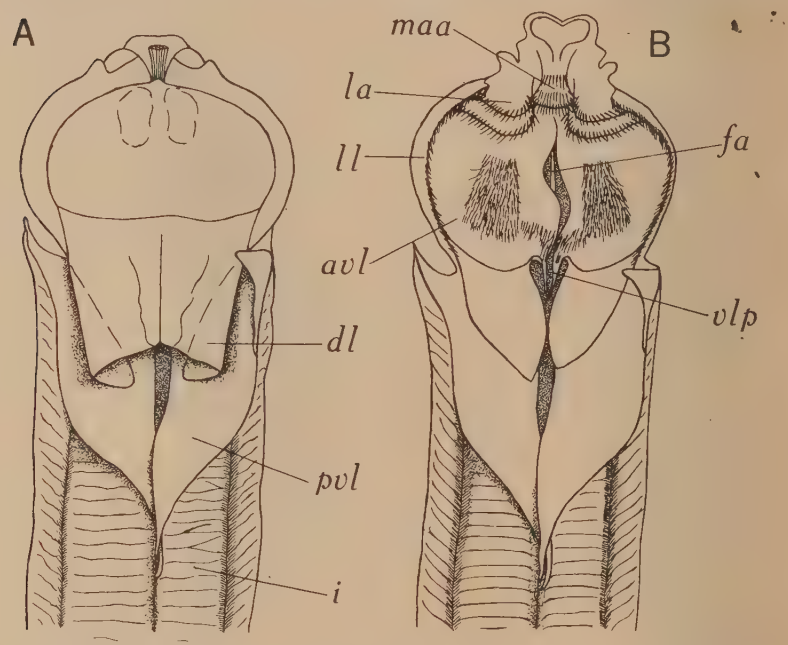
distal attachment dorsal to the antennal fossæ. On removal of these muscles others will be seen attached to the ventral wall of the gut and to the "median tooth" of Hewitt's description.

(ii) *Internal Structure.*

In the description of the foregut that follows the terminology employed by Hewitt has been retained as far as possible, to avoid further confusion. It has been essential to delete the term "tooth," which is very misleading, as are also the terms "cardiac" and "pyloric" when applied to this animal, the use of which suggests a division into two parts comparable with the state of affairs in other Malacostraca. No such division can be observed in the foregut of Ligia. For the term "tooth" the word "ampulla" has been substituted for all cushion-like projections, hollow

and covered with a thin layer of chitin. The term lamella has been retained throughout.

From the anterior regions of the side walls of the foregut arises a pair of large, bilobed projections, the lateral ampullæ (*l.a.*, Text-Figs. 2, 3, 4, 6, and 8), meeting above the opening to the œsophagus. Anterior to these and hidden behind them is a pair of very small antero-lateral ampullæ



Text-Fig. 2.—A, Foregut from dorsal surface with intestine cut open along mid-dorsal line. B, same with dorsal lamella removed to show underlying structures.

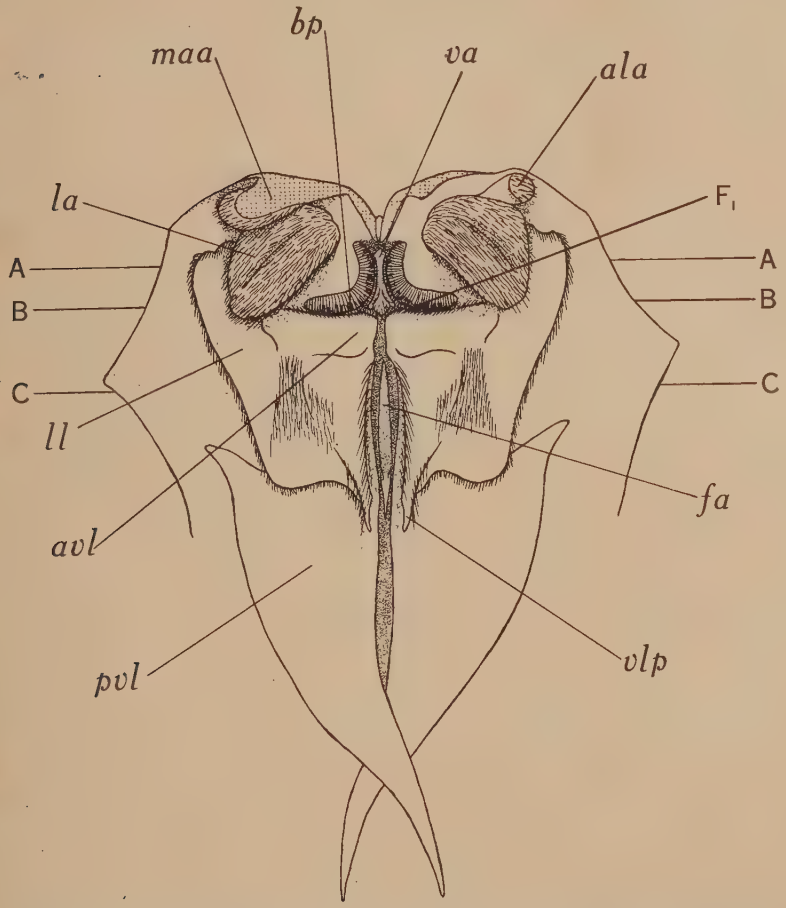
a.v.l., anterior ventral lamella; *d.l.*, dorsal lamella; *f.a.*, filter apparatus; *i.*, intestine; *l.a.*, lateral ampulla; *l.l.*, lateral lamella; *m.a.a.*, median anterior ampulla; *p.v.l.*, posterior ventral lamella; *v.l.p.*, projections from anterior ventral lamellæ.

(*a.l.a.*); in the mid-line of the anterior wall arises a single large median anterior ampulla (*m.a.a.*), while in the floor of the foregut lies a single median projection, the ventral ampulla (*v.a.*). These are all provided with strong, backwardly projecting bristles, and on contraction of the muscles of this region these ampullæ meet and effectively close the entrance to the œsophagus.

From the floor of the foregut and directly behind the ventral ampulla arises a pair of plates, the anterior ventral lamellæ (*a.v.l.*) forming to all appearances the actual floor which here appears to have a median ridge.

This ridge is formed by the approximated inner edges of these plates which rise towards the mid-line, each bearing a pointed projection (*v.l.p.*).

The anterior ventral lamellæ fuse laterally with the lateral lamellæ (*l.l.*), a pair of plates arising from the antero-lateral walls of the chamber,



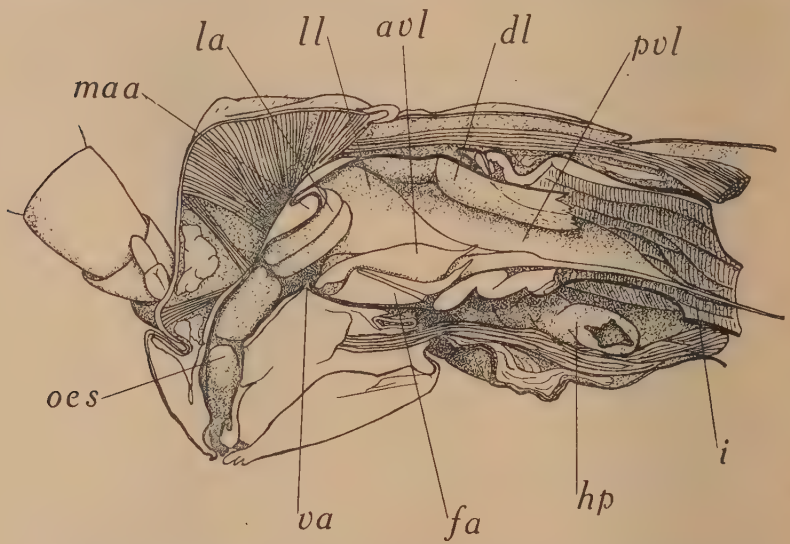
TEXT-FIG. 3.—Foregut opened along mid-line of the dorsal and anterior walls as far as the opening of the œsophagus. The two halves of the dorsal lamella are drawn apart, giving a flattened view of the main structures of the interior. The right half of the median anterior ampulla has been removed to disclose the small antero-lateral ampulla.

AA, BB, and CC indicate the lines through which the sections shown in Fig. 8 are drawn.

a.l.a., antero-lateral ampulla ; *a.v.l.*, anterior ventral lamella ; *b.p.*, bristle plate ; FI., Filter I ; *f.a.*, filter apparatus ; *l.a.*, lateral ampulla ; *l.l.*, lateral lamella ; *m.a.a.*, median anterior ampulla ; *p.v.l.*, posterior ventral lamella ; *v.a.*, ventral ampulla ; *v.l.p.*, projection from anterior ventral lamella.

beneath the lateral ampullæ and proceeding backwards and downwards as a flap attached ventrally. They are provided along the free dorsal edge with strong backwardly projecting bristles. The anterior ventral lamellæ, moreover, fuse posteriorly with a pair of long plates, the posterior ventral lamellæ (*p.v.l.*) which taper gradually to end in the intestine, projecting considerably beyond the apparent limits of the foregut.

If the ventral lamellæ are drawn apart a wedge-shaped projection is seen arising from the true floor of the foregut, widening posteriorly and



Text-Fig. 4.—Sagittal section through cephalon and first free thoracic segment, slightly to left of mid-line to include filter apparatus, showing internal structure of the right side. The cut edges of the alimentary canal are heavily outlined in black.

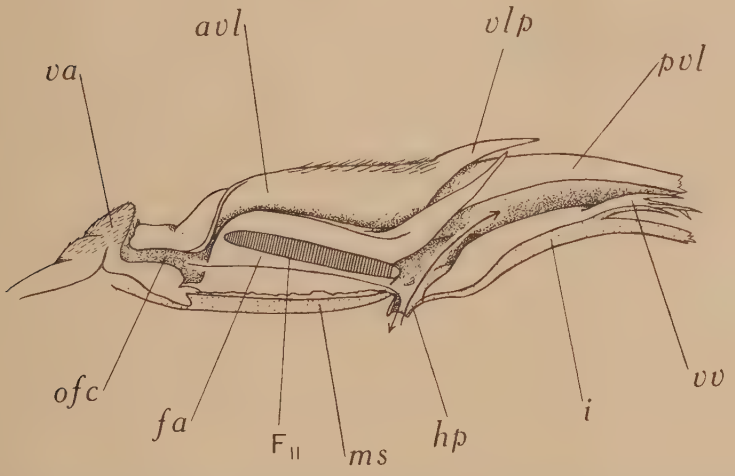
a.v.l., anterior ventral lamella; *d.l.*, dorsal lamella; *f.a.*, filter apparatus; *h.p.*, hepato-pancreas; *i.*, intestine; *l.a.*, lateral ampulla; *l.l.*, lateral lamella; *m.a.a.*, median anterior ampulla; *oes.*, œsophagus; *p.v.l.*, posterior ventral lamella; *v.a.*, ventral ampulla.

bearing on its postero-dorsal surface a finely pointed projection, similar to those of the ventral lamellæ, between which it is inserted. This structure, for want of a better name, may be called the filter apparatus (*f.a.*). It is, presumably, the "median ventral tooth" of Hewitt. Nothing appears more certain than that it is not a tooth; a more detailed description of this apparatus appears below.

There remains one more lamellar structure to be mentioned, a simple projection from the dorsal surface of the foregut, arising almost directly over the junction of the anterior and posterior ventral lamellæ and

proceeding backwards to project freely into the lumen of the intestine, the dorsal lamella (*d.l.*). This is, apparently, without setose armature.

On the floor of the foregut and on either side of the elongate ventral ampulla lies a crescentic row of bristles, fused at their bases to form a plate (*b.p.*), but with their distal ends lying against the sides of the ventral ampulla in the mid-line and against the anterior ventral lamellæ behind. Beneath these bristles and on each side of the ventral ampulla is a channel running posteriorly under the anterior ventral lamellæ.



TEXT-FIG. 5.—Section through the foregut, slightly to left of mid-line, showing the filter apparatus and associated structures. The arrows indicate the course of food passing into the hepato-pancreas and the path taken by the secretion when forced out.

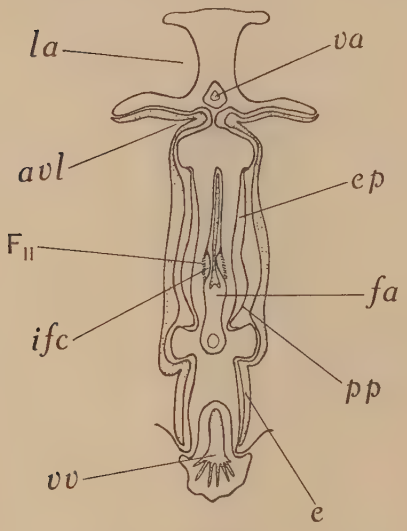
a.v.l., anterior ventral lamella ; *f.a.*, filter apparatus ; FII., Filter II ; *h.p.*, junction of hepato-pancreas with intestine ; *i.*, intestine ; *m.s.*, muscle sheath ; *o.f.c.* outer filter channel ; *p.v.l.*, posterior ventral lamella ; *v.a.*, ventral ampulla, *v.l.p.*, projection from anterior ventral lamella ; *v.v.*, ventral valve.

This will best be seen in Text-Figure 8. It constitutes Filter I. The occlusion of this channel (*o.f.c.*) between the bristle plates and the anterior ventral lamellæ is effected by a row of hairs which project forwards and interlock with those of the bristle plates.

Beneath the anterior ventral lamellæ the channel is divided into two by the filter apparatus (*f.a.*). As shown in Text-Figures 5, 6, and 8, this is wedge-shaped in two directions. It is broader at the base than at its dorsal edge and widens from in front backwards. On either side and parallel with the dorsal edge lies a groove covered with strong bristles attached to the ventral surface and free dorsally. This groove ends blindly anteriorly, opening over the entrance to the hepato-pancreas posteriorly. This is

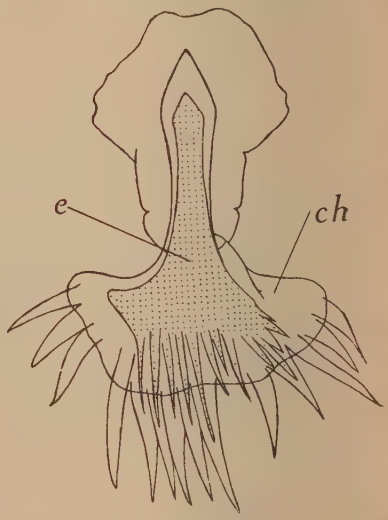
Filter II. Rehorst describes similar structures in the foregut of Asellus and the filters and filter channels of Ligia are directly comparable with those of Asellus, for which reason I have used the names given by him.

Finally, there is a structure, the ventral valve (*v.v.*, Text-Figs. 5, 6, and 7), attached to the ventral wall of the intestine and bearing against the lower surface of the posterior ventral lamellæ. We have now a space stretching from the ventral ampulla anteriorly to the ventral valve



Text-Fig. 6.—Semi-diagrammatic horizontal section through the filter apparatus showing its relationship to the surrounding structures.

a.v.l., anterior ventral lamella; *e.*, epithelium; *e.p.*, "elastic pad" of Rehorst; *f.a.*, filter apparatus; FII., Filter II; *i.f.c.*, inner filter channel; *l.a.*, lateral ampulla; *p.p.*, perforated plate; *v.a.*, ventral ampulla; *v.v.*, ventral valve.



Text-Fig. 7.—The ventral valve. *e.*, epithelium; *ch.*, chitin.

posteriorly which can be completely closed to any but liquid substances, all the possible entrances being guarded by strong hairs.

The chitinous lining of the anterior ventral lamellæ facing Filter II is composed of a double layer, the outer in contact with the filter being provided with very fine, upwardly projecting hairs, the inner being a plate with many minute holes, the two layers being connected by a network of fine fibres. Behind this inner plate lies the ordinary epithelial tissue, but at a little distance, thus enclosing a second space. This, however, is probably an artifact due to fixation since this space is found throughout

where chitin forms the lining of the foregut. (See Text-Figures 6, 8, and 9.)

Below is a list of the parts of the foregut as named by Hewitt in the first column; the terminology employed in this paper in the second, and that used by Rehorst for Asellus, as far as it is comparable, in the third.

Lateral cardiac tooth.	Lateral ampulla.	Laterale.
Antero-lateral tooth.	Antero-lateral ampulla.	—
Median anterior tooth.	Median anterior ampulla.	—
Ventral cardiac tooth.	Ventral ampulla.	Y-shaped piece.
Ventro-lateral tooth.	Anterior ventral lamella.	Infero-Laterale.
Median ventral tooth.	Filter apparatus.	Infero-Medianum.
Lateral cardiac lamella.	Lateral lamella.	Dorsal branch of the Laterale.
Dorsal lamella.	Dorsal lamella.	Supero-Medianum.
Ventro-lateral pyloric lamella.	Posterior ventral lamella.	Intero-Laterale.

Actually Rehorst includes his Y-shaped piece as part of the Infero-Laterale; he also confines the term "outer filter channel" to that portion of the cavity enclosed between the two ventral lamellæ which lies below Filter II, the whole space being termed the "storeroom."*

(*c*) The Hepato-pancreas.

This consists of three pairs of tubules lying along the alimentary canal which they partially enclose. Their external morphology has been fully described by Hewitt, but a little more must be said about their internal structure. Reference to Plate I, Figures 4 and 5, will show that there are small and large cells, as mentioned by many workers on the histology of this organ in the Isopods. The cell walls are distinct and homogeneous, the contents granular with many small vacuoles, the significance of which cannot be entered into here. Each cell contains a nucleus, and nucleoli are present in most.

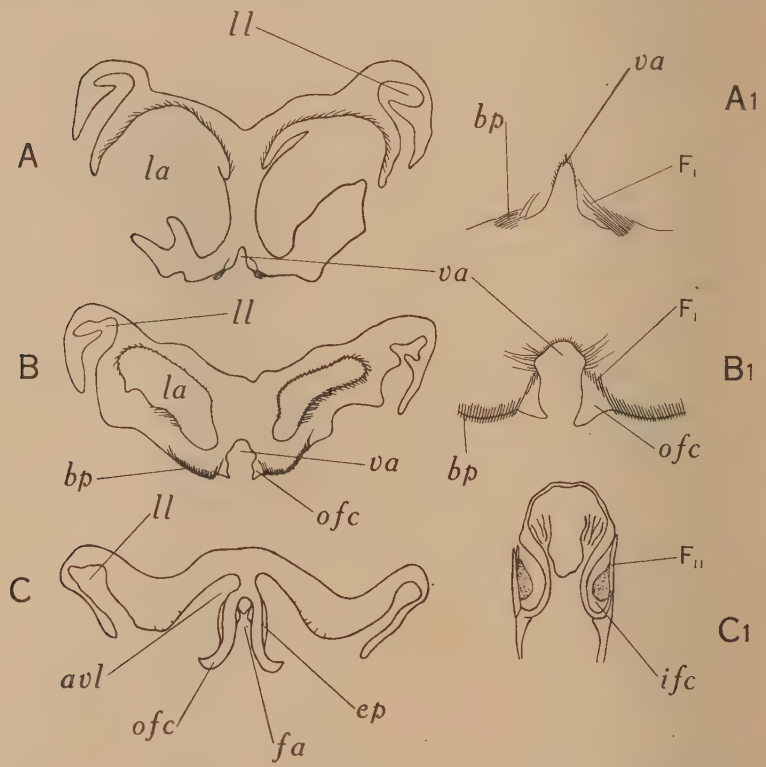
It is the opinion of Nusbaum-Hilarowicz (1920) that the small cells are young stages of the large ones which, as they become mature, break up, liberating their contents into the lumen and thus providing the secretion. In this respect Nusbaum-Hilarowicz supports the view held by Murlin (1902) though Weber (1880) thinks that they are absorbing and secreting cells respectively.

(*d*) The Intestine

As McMurrich (1896) has shown, the portion of the alimentary canal extending from the point of entry of the hepato-pancreatic glands to the

* I wish to thank Dr. H. G. Jackson for details of the technique employed by him in dissecting the head of Ligia.

rectum, usually termed the midgut, is, in Isopods, the anterior portion of the proctodæal invagination and cannot, therefore, be justly termed midgut. This statement is supported by my observations on Ligia in that the whole of this region is lined throughout by chitin. This layer of chitin is, as McMurrich has shown, double, but whereas this author

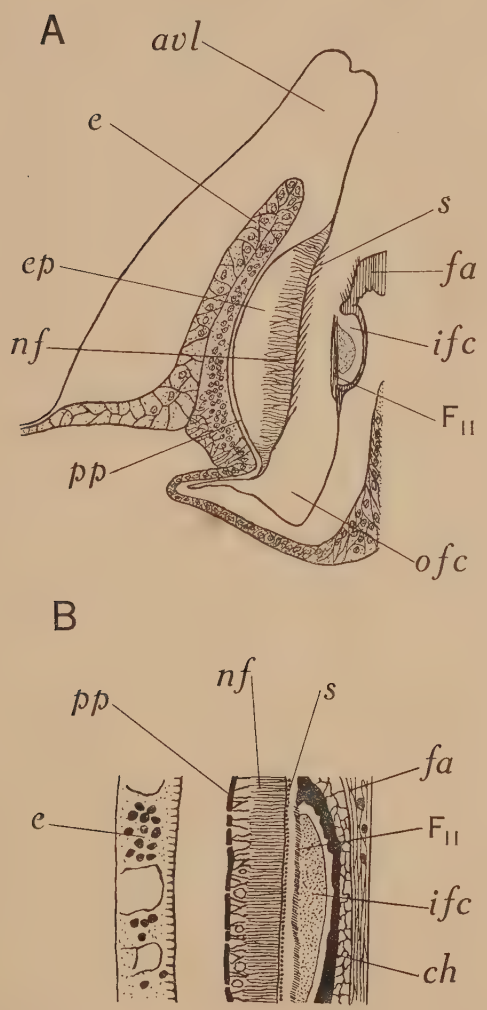


TEXT-FIG. 8.—Semi-diagrammatic transverse section through foregut, A and B passing through bristle plates, ventral ampulla and Filter I; C, passing through filter apparatus and Filter II, as indicated by AA, BB and CC in Fig. 3. A1, B1 and C1, enlargements of portions of A, B, and C.

a.v.l., anterior ventral lamella; *b.p.*, bristle plate; *e.p.*, "elastic pad" of Rehorst; *f.a.*, filter apparatus; FI., Filter I; FII., Filter II; *i.f.c.*, inner filter channel; *l.a.*, lateral ampulla; *l.l.*, lateral lamella; *o.f.c.*, outer filter channel; *v.a.*, ventral ampulla.

describes both layers as homogeneous, in Ligia the inner layer appears to be vacuolated (Plate I, Figs. 1, 2, and 3).

Murlin (1902, p. 310) describes the presence of minute pores which perforate the chitinous lining of the lumen of the "midgut" in land Isopods. He does not illustrate these pores though he states that they are easily



TEXT-FIG. 9.—A, semi-diagrammatic representation of transverse section through ventral lamella and filter apparatus, showing relationship between Filter I and "elastic pad" of Rehorst. B, semi-diagrammatic representation of horizontal section through same region, showing network of fibres, perforated plate and space behind plate.

a.v.l., anterior ventral lamella; *ch.*, chitin; *e.*, epithelium; *e.p.*, "elastic pad" of Rehorst; *f.a.*, filter apparatus; FII., Filter II; *i.f.c.*, inner filter channel; *n.f.*, network of fibres; *o.f.c.*, outer filter channel; *p.p.*, perforated plate; *s.*, setæ of "elastic pad."

demonstrated in the fresh intima found in a moulted posterior portion of the animal. Schonichen (1898) also found such pores in the Oniscidæ and Asellidæ. Examination of similar material and also of sections has failed to establish the presence of such pores in Ligia, but as will be seen from Plate I, Figures 2 and 3, the chitin lining the epithelium of the intestine is of a complicated nature.

It is composed of two layers of unequal thickness making up a total of surprising thickness for such a region. The inner layer, comprising about three-quarters of the total thickness, is of a loose spongy texture containing cavities, many of which are permeated by cytoplasm. The outer layer is very thin but apparently homogeneous and quite imperforate.

In the absence of any pores in these layers Ligia is in perfect agreement with McMurrich's description for Armadillidium, Porcellio, Oniscus, and *Idothea robusta*.

The epithelium underlying the chitinous layer is devoid of cell boundaries and forms a syncytium. "Supportive fibres" arise from both this layer and the basement membrane and run towards the opposite sides, representing all that is present of cell walls.

The infoldings of the intima, which project into the syncytial layer, are very regular and viewed from the surface under low magnification give the impression of an epithelium composed of large, very regularly arranged cells, each containing a large nucleus. This impression is heightened by the arrangement of the muscle fibres on the outside which form a network, each strand lying in a groove on the surface. No trace of a typhlosole can be seen in Ligia.

(*e*) The Rectum.

The terminal portion of the alimentary canal is a short region with very much folded walls, well supplied with muscles. It is separated from the intestine by a sphincter. The epithelium in this region is a syncytium continuous with that of the intestine, but much thinner.

3. PHYSIOLOGY OF THE GUT.

The structure and function of what we now term the hepato-pancreas has been investigated in Isopods and other Crustacea by Weber (1880), his work being done largely on Asellus. Murlin (1902) and Nusbaum-Hilarowicz (1920) have dealt similarly with the land Isopods, the latter going into much histological detail, while Patrick (1926) alone has investigated the cells of the hepato-pancreas of Ligia. Gelderd (1906) concluded from his research on the function of the foregut that the "cardiac chamber" primarily masticates the food particles "as an auxiliary to the mouth pieces" and secondarily it "acts as a sieve or filter for the further

retention in the cavity of such particles of food that have not been sufficiently divided." The "pyloric chamber," he says, has the function "of mixing the already masticated food with the ferments of the digestive gland. This is brought about by the action of the spines and hairs upon the pyloric pieces, when the chamber is put into movement by the muscles."

Hewitt (1907), speaking of Ligia, says: "The stomach forms an efficient mill for triturating the miscellaneous substances upon which the animal feeds." Tait (1917), in describing the structure of the foregut of Glyptonotus, says: "The use of the term 'gizzard' (chosen apparently as an improvement on the older and admittedly unsuitable term 'stomach') is in itself misleading, for the name suggests that the function of the organ is to triturate the food. The idea is disposed of by the condition of the ingesta discovered in the midgut of the dissected specimens. When the food had consisted of amphipods these were found, according to size, almost intact or cut into longitudinal blocks of about $\frac{1}{4}$ inch length." He goes on to say: "The cutting had evidently been done by the incisor processes of the mandibles, the length of the blocks corresponded roughly to the reach of these processes from the position of abduction to that of adduction, and the food had evidently been 'bolted' without the occurrence of any further process of comminution in the vestibule." This latter is the term he employs in place of the previous misleading terms. He is further of the opinion that the foregut is "merely a propelling mechanism." While, in my opinion, the above description of the supposed method of feeding of Glyptonotus applies equally well to Ligia, since I have found the contents of the intestine to consist of equal sized particles of algæ, yet it will be shown that the foregut is more than a mechanism for propulsion. I have, moreover, through the kindness of Professor D. M. S. Watson, been able to dissect a specimen of Glyptonotus and find that in main principles the foregut resembles that of Ligia very closely. Hewitt, in using the term "tooth," admits its inadequacy, but still assigns to it a triturating function, which rather nullifies the admission. None of the structures termed by him "teeth" are in any way comparable with the hard chitinous teeth of the Decapods.

Assuming, then, that no further mastication is performed after the food has passed the mouth parts, it will pass up the œsophagus as a "mush" and will enter the foregut between the bristle plates and ventral ampulla below, and the lateral and median anterior ampullæ above. Here, by contraction of the muscles enclosing this part of the vestibule (Text-Fig. 1), to use Tait's term, the liquid portion of the food will be squeezed through Filter I carrying with it only very fine particles. The same motion which compresses the food will tend to force it back into the larger cavity of the foregut, by virtue of the backwardly projecting hairs with which

it will be in contact all round. Imagine now a contraction of the muscles, shown in Text-Figure 1 running forward from the lateral lamellæ, to occur simultaneously with a contraction of the muscle sheath across the ventral, convex surface of the foregut. The result would be the drawing apart of the ventral lamellæ and the pushing up of the filter apparatus. Thus the secretion from the hepato-pancreas would be liberated into the cavity of the vestibule and mixing would also be effected.

Moreover, on the return of the parts to their normal position, a quantity of partly digested food would be caught between each ventral lamella and the filter apparatus, and the liquid portion squeezed through Filter II into the inner filter channel, whence it would pass into the hepato-pancreas along with the liquid forced through Filter I into the outer filter channel.

This process is further aided by the rhythmical contractions of the hepato-pancreatic tubules. These were observed in the living animal, through the transparent ventral surface of a recently moulted specimen, to consist of waves passing forwards along the tubules expelling the contents. On the relaxation of the muscles of the tubules, liquid was observed to return into the lumen. The contraction of the tubules probably coincides with that of the muscle sheath which, as we have seen above, separates the ventral lamellæ and elevates the filter apparatus. Thus is the secretion from the glands passed into the foregut and intestine. With the return of the parts of the foregut to their normal position and the relaxation of the muscles of the tubules, liquid forced through the filters into the filter channels is drawn into the hepato-pancreas. The entrance from the intestine is automatically closed by the action of the ventral valve. Thus the liquid products of digestion may be absorbed in the tubules of the hepato-pancreas. The more solid portions of the food, mixed with the digestive enzymes, will pass into the intestine, where further digestion and absorption will occur.

It will be seen from the above description of the assumed functioning of the foregut that no time is wasted with mastication, the whole principle being one of extracting as quickly as possible the most readily obtainable nourishment. It is suggested that this may be correlated with the limited period during which the animal feeds, as described above, in which case it would be expected also that the enzymes which break down the food would be very powerful, and this will be shown to be the case.

Rehorst, apparently, assumes the filtering action to be quite passive, at least in Filter I, and to the surfaces which come into contact with the filters and are themselves setose, he assigns primarily the function of cleaning the filters and keeping them free from large particles. This may easily be an additional function in Ligia. Further, he assumes that the space enclosed between the double layer of chitin lining the opposing walls of the ventral lamellæ is filled with liquid, forming an elastic pad

which, pressed against Filter II, would separate the outer filter channel from the space above. He makes no mention of perforations in the inner wall of the pad, possibly they are absent in Asellus, but such a function as he suggests for this pad, besides being superfluous, would be rendered difficult in Ligia unless liquid could be forced in at will. The function of this part remains somewhat obscure.

4. COMPARISON OF THE FOREGUT OF LIGIA WITH THAT OF THE DECAPODS.

As may be seen from the work of Jordan (1904), Williams (1907), and Yonge (1924), the foregut in the Decapods is divided into two parts, the cardiac and the pyloric, to each of which are assigned definite functions.

The cardiac portion is a sac serving mainly for the reception of food and contains the masticatory ossicles which triturate the food and assist in the mixing of the food with the secretion from the hepato-pancreas. The food then passes into the pyloric region whence the liquid portion and fine particles pass through the filter into the hepato-pancreas, the larger particles passing above the press into the midgut.

Whereas the Decapods merely tear their food with their mouth parts and pass it into the foregut for complete mastication and digestion, Ligia, as has been seen, not being provided with a triturating mechanism in the foregut, cuts up its food with the mouth parts and then depends on the great power of its digestive enzymes to complete the process in the foregut and intestine. Both are provided with means of limiting the size of the particles entering the hepato-pancreatic tubules, and both the valves and the plates of the Decapods and the ventral valve and lamellæ of Ligia prevent the blockage by food particles of the exit from the tubules. Further, in the action of the ventral lamellæ working in conjunction with the filter apparatus, there is in Ligia a mechanism comparable with the press of the Decapods.

5. DIGESTION.

(*a*) The pH of the Gut.

In Table I will be found the pH of the different regions of the gut of normally feeding animals, of starved animals (48 hrs.), and of those whose pH had been estimated at different periods after feeding following on a period of starvation of 48 hours or more.

From Table I it will be seen that the pH of the gut varies very slightly from one end to the other, whether the animals are starved or feeding. As a result of feeding after starvation the pH of the intestine

was found to be slightly higher than the contents of the hepato-pancreas and slightly lower than that in the same region of a starved animal. This was the same whether estimated ½ hr. or 4 hrs. after feeding.

For the estimation of pH on the small quantities of liquid found in the gut of this animal the method employed was that described by Wigglesworth (1927, p. 792), and used by him in similar work on the cockroach.

TABLE I.

Showing pH of Gut under Different Conditions.

ANIMALS ALL FEEDING.

No.	Intestine.	Rectum.
1.	6·0	6·2
2.	6·2	5·9
3.	6·7	—
4.	6·3	—
Mean.	6·3	6·05

ANIMALS STARVED FOR 48 HOURS.

No.	Foregut.	Intestine.	Rectum.
1.	—	6·05	6·0
2.	6·3	6·55	6·2
3.	6·3	6·45	6·2
4.	6·4	6·4	6·5
5.	6·3	6·55	6·1
6.	6·1	6·25	5·8
7.	6·25	7·2	6·3
Mean.	6·27	6·49	6·16

STARVED ANIMALS BEFORE AND AFTER FEEDING.

No.	Period of Starvation hours.	Intestine.	Hepato-pancreas.
1.	48	6·4	6·0
2.	60	6·3	6·0

No.	Period of Starvation hours.	Time after feeding hours.	pH of Intestine.
1.	60	½	6·2
2.	60	4	6·2
Mean.	–	–	6·2

(*b*) The Digestive Enzymes.

The enzymes secreted by the cells of the hepato-pancreas are passed into the gut by rhythmical contractions brought about by the network of muscles on the outside, described by Pump (1914).

An extract was made from the hepato-pancreatic tubules of a large number of animals ground up with silver sand and extracted on ice in distilled water for 48 hours.

This was then filtered and made up to a 10% solution based on the weight of tubules and secretion obtained, the whole being kept under toluol continuously. The pH of this extract was 5·9. Table II shows the result of incubation of various substances with this extract.

TABLE II.

5 c.c. substrate ; 5 c.c. extract ; toluol. Controls boiled. Temperature 30° C.

Presence of glucose determined by Fehling's solution, and of di-saccharides by Barfoed's solution. Fatty acids produced by digestion of fat titrated against N/100 NaOH.

— indicates no action ; ± indicates a trace ; + indicates positive action ; ++ indicates extensive action.

Substrate.	Experiment after				Control after			
	24 hrs.	48 hrs.	4 days.	10 days.	24 hrs.	48 hrs.	4 days.	10 days.
Starch 1%	++	++	..	..	±	±	..	..
Glycogen (Saturated)	+	+	..	..	±	±	..	..
Sucrose 5%	++	++	..	..	–	–	..	..
Raffinose 1%	++	++	..	..	–	–	..	..
Inulin 1%	–	–	–	..	–	–	–	..
Maltose 2%	+	+	..	..	–	–	..	..
Lactose 2%	–	–	–	..	–	–	–	..
Cellulose	–	–	–	–	–	–	–	–
Amygdalin 1%	+	+	..	..	–	–	..	..
		CN evov.						
Salicin 1%	+	+	..	..	–	–	..	..
Fibrin	Digestion complete 24 hours. Positive reactions with Millon's Reag. and Br. water.				–	–	–	..
Olive Oil	..	2·15 c.c.	2.0 c.c.	..	..	1·9 c.c.	1·88 c.c.	..

From the above Table it will be seen that most carbohydrates are readily split up, the exceptions being Lactose, Inulin, and Cellulose, while the protease is exceptionally strong, digestion of a large piece of fibrin being complete within 24 hours. A lipase is also present.

In order to discover if any secretion was liberated by the intestine a number of these were taken, split open, and washed carefully in water and ground up as were the tubules of the hepato-pancreas, being extracted in 50% glycerin for 48 hours. Table III shows the result of this experiment, from which it will be seen that after 24 hours glycogen and sucrose both showed a trace of reduction and starch after 48 hours.

TABLE III.

5 c.c. substrate with 5 c.c. extract and toluol; incubated at 30° C.
— indicates no action; ± indicates a trace; + indicates positive action. Tests as before.

Substrate.	Experiment after 24 hours.	Experiment after 48 hours.	Control after 24 hours.	Control after 48 hours.
Starch 1%	±	+	—	—
Glycogen (Sat)	±	±	±	±
Sucrose 5%	+	+	—	—
Raffinose 1%	—	—	—	—
Inulin 1%	—	—	—	—
Maltose 2%	±	±	—	—
Lactose 2%	—	—	—	—
Cellulose	—	—	—	—
Amygdalin 1%	—	—	—	—
Salicin 1%	—	—	—	—
Fibrin	—	—	—	—
Olive Oil	..	0·35 c.c.	..	0·30 c.c.

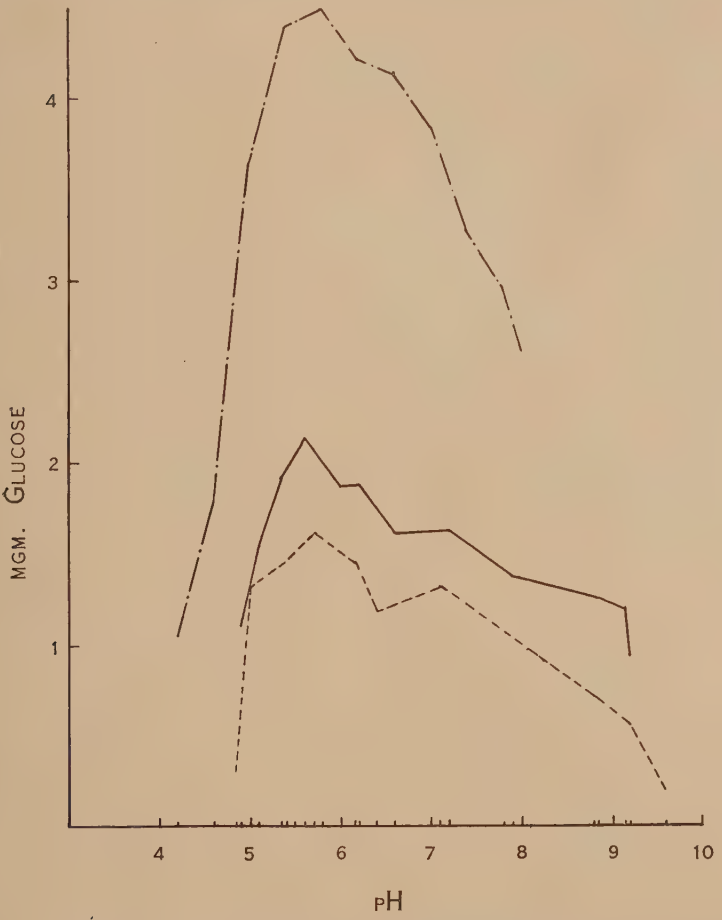
The original sample of glycogen, however, was found to contain a certain amount of reducing sugar, while the trace of reduction in the other two can be explained by the great ease with which they are reduced by the secretion from the hepato-pancreas, combined with the difficulty experienced in washing the intestines completely free from that secretion. It can safely be assumed that there are no enzymes secreted by the epithelium of the intestine which can act on carbohydrates, proteins or fats. Thus the whole of the digestion is performed by the enzymes present in the secretion from the hepato-pancreas.

(*c*) The Sucroclastic enzymes.

Since algæ form the normal food of this animal, it was thought advisable to investigate the possibility of enzymes being present which would reduce pentosans and cellulose. Accordingly tests were made on Gum Arabic, Agar Agar, and Pectin. No reducing substances were found in any case after 24 hours' incubation at 27° C., using 10% extract.

For the detection of a possible cellulase other tests were applied. A small piece of clean Fucus was placed in the extract and incubated for several days. At the end of that time microscopic examination showed that the contents of only those cells whose walls had been cut through were digested. Further, hand sections were cut and mounted on a slide in the actual secretion from the hepato-pancreas, the cover glass being sealed and the whole left in a warm, constant temperature for several

days, being examined from time to time for dissolution of the cell walls. None, however, was observed. In tests on filter paper and cotton wool incubated for some time with the extract, Fehling's solution failed to demonstrate the presence of any reducing sugar. Thus it will be seen



TEXT-FIG. 10.—Graph showing pH optimum and range of activity for amylase ——; invertase ——.——; glycogenase – – – – –.

that this animal, though its main diet consists of Fucus and other vegetable matter, is quite unable to digest either cellulose or the pentosans contained in such food. Yonge (1927) failed to find a cellulase in extracts of the wood-boring Isopod *Limnoria lignorum*.

A series of experiments was carried out to determine the pH optima of

the various sucroclastic enzymes. The effect of the change of pH upon the activity of the amylase, invertase and glycogenase is shown in Tables IV, V, and VI respectively, below and in Text-Figure 10.

In these experiments Clark and Lub's Buffer Solutions were used, at first, for providing a known pH. These, however, were found in some cases to be altered by the addition of the extract and did not give true readings. The method then resorted to was that of adding a certain quantity of acid or alkali and/or water to the extract and finding the pH of the resultant mixture.

MacLean's blood sugar method was used throughout in estimating the amount of glucose. Briefly, this is as follows: The proteins are precipitated from the sample by heating with acid sodium sulphate and adding dialysed iron to the hot liquid. The mixture is cooled and filtered and to the filtrate is added an excess of alkaline copper iodide solution. This is then boiled for a definite length of time, during which an amount of copper is reduced equivalent to the amount of glucose present in the sample. The iodine is liberated from the excess alkaline copper iodide by the addition of acid and titrated with sodium thiosulphate in the usual way. The amount of iodine present in the total amount of copper iodide solution added is also determined directly, the difference giving the amount of copper iodide reduced by the glucose, from which the amount of glucose present can be determined.

TABLE IV.

pH Optimum for Amylase.

1% starch solution, 1 c.c. 10% enzyme extract, 1 c.c. Acid or alkali and/or water to 2 c.c.

Incubated for 2½ hours. Temperature, 28° C.

No.	Acid		Alkali		H_2O	pH.	Thio. ·01 N	Glucose
	c.c.	N.	c.c.	N.	c.c.		c.c.	mgm.
1.	1·0	·01	–	–	1·0	4·9	5·43	1·11
2.	·5	·01	–	–	1·5	5·1	4·37	1·53
3.	·3	·01	–	–	1·7	5·35	3·49	1·91
4.	–	–	–	–	2·0	5·65	2·96	2·13
5.	–	–	·25	·01	1·75	6·0	3·58	1·87
6.	–	–	1·0	·01	1·0	6·2	3·58	1·87
7.	–	–	2·0	·01	–	6·6	4·20	1·61
8.	–	–	·3	·1	1·7	7·2	4·16	1·62
9.	–	–	·45	·1	1·55	7·9	4·78	1·37
10.	–	–	1·0	·1	1·0	8·85	5·11	1·25
11.	–	–	1·5	·1	·5	9·15	5·27	1·18
12.	–	–	2·0	·1	–	9·2	5·83	0·94

TABLE V.

pH Optimum for Invertase.

5% sucrose solution, 2·5 c.c. 10% enzyme extract, 2·5 c.c. Buffer solution, 5 c.c.

Incubated for 2 hours. Temperature, 32° C.

No.	pH.	Thio. ·01 N. c.c.	Glucose mgm.
1.	4·2	7·60	1·05
2.	4·6	6·60	1·79
3.	5·0	4·36	3·63
4.	5·4	3·46	4·39
5.	5·8	3·35	4·48
6.	6·2	3·66	4·22
7.	6·6	3·87	4·13
8.	7·0	4·12	3·83
9.	7·4	4·78	3·27
10.	7·8	5·15	2·96
11.	8·0	5·60	2·60

Note.—In the above table the amount of glucose represented is, in some cases, greater than the amount that can be estimated by the amount and strength of reagents advised by MacLean. In estimating glucose in this experiment half the quantity was taken and the result doubled.

TABLE VI.

pH Optimum for Glycogenase.

Saturated solution of Glycogen, 3 c.c. 10% enzyme extract, 1 c.c. Acid or alkali and/or water to 2 c.c.

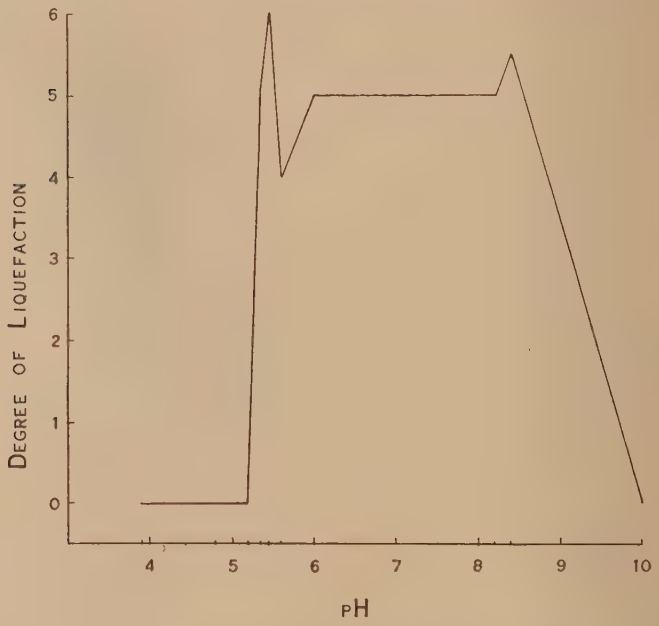
Incubated for 70 minutes. Temperature, 32° C.

No.	Acid		Alkali		H_2O	pH.	Thio. ·01 N.	Glucose
	c.c.	N.	c.c.	N.	c.c.		c.c.	mgm.
1.	2·0	·01	–	–	–	4·85	7·16	0·3
2.	1·0	·01	–	–	1·0	5·0	4·97	1·31
3.	·5	·01	–	–	1·5	5·4	4·54	1·46
4.	–	–	–	–	2·0	5·7	4·20	1·61
5.	–	–	1·0	·01	1·0	6·15	4·58	1·45
6.	–	–	2·0	·01	–	6·4	5·25	1·19
7.	–	–	·35	·1	1·65	7·1	5·01	1·32
8.	–	–	·4	·1	1·6	8·8	6·46	0·71
9.	–	–	·6	·1	1·4	9·2	6·86	0·56
10.	–	–	1·0	·1	1·0	9·6+	7·62	0·20

There is a range of activity between pH 5 and pH 7 in the case of all three sucroclastic enzymes, with the optimum lying between pH 5·65 and pH 5·8.

(*d*) The Proteoclastic Enzymes.

These are very powerful, so much so that it was necessary to reduce the proportion of extract to substrate very considerably in order to prevent the digest from acting too quickly. Table VII and Text Figure 11



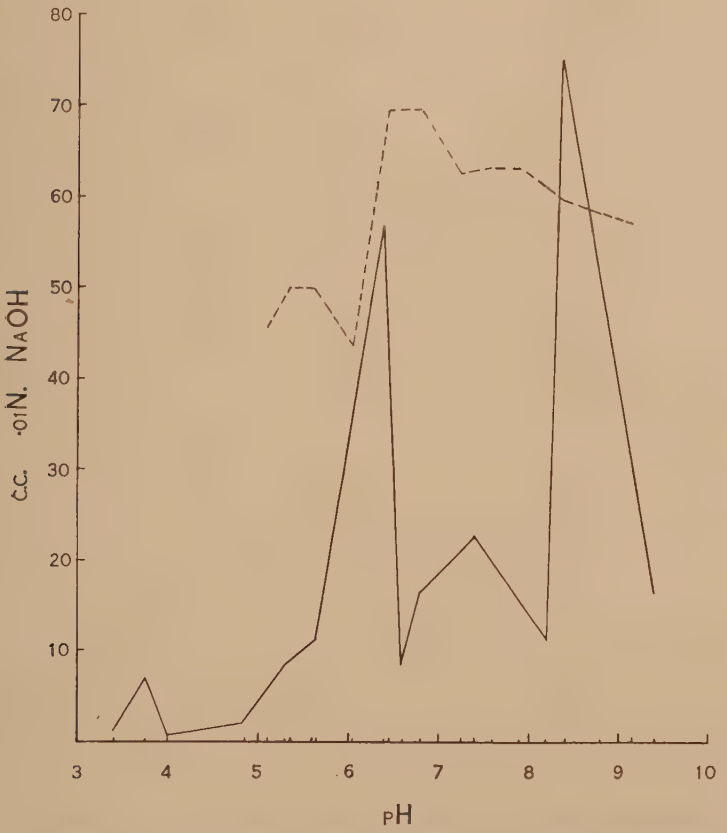
Text-Fig. 11.—Graph showing activity of protease, with change in pH, on the liquefaction of gelatine.

represent the results of an experiment to determine the effect of change in hydrogen ion concentration on the liquefaction of gelatine, i.e. the conversion of proteins to soluble polypeptides.

In this Table the degree of liquefaction is expressed by the figures of an arbitrary scale used by Yonge (1926a) who quotes Dernby, as follows :—

0 Completely solid.
1 Solid, but small pieces may be torn off by strong shaking.
2 Solid, but the surface moves somewhat when the tubes are shaken.
3 Soft.
4 Half liquid.
5 Almost liquid.
6 Entirely liquid.

From these it will be seen that the range of activity extends from pH 5·35 to pH 5·85, with an optimum about pH 5·45 and a second at pH 8·4. To determine the quantities of amino acids produced these were estimated by titration with alkali after treatment of the digest with 90% alcohol. This proved an unsatisfactory method owing to the precipitation of proteins which masked the end point. Sørensen's formaldehyde titration was employed with more success on digests with blood fibrin. The results of this experiment are given in Table VIII and Text-Figure 12. Two optima were again found, the first at pH 6·4 and the second at pH 8·4.



TEXT-FIG. 12.—Graph showing optimum pH and range of activity for protease on blood fibrin ——; and on peptone – – – – –.

TABLE VII.

pH Optimum for the Protease by the Liquefaction of Gelatine.

10% gelatine solution, 5 c.c. and 1 c.c. of the following mixture: 0·5% enzyme extract in sea-water, 5 c.c., acid or alkali and/or water to 5 c.c. Temperature, 32° C.

No.	Acid		Alkali		H_2O	pH	Degree of liquefaction after given number of hours.							
	c.c.	N.	c.c.	N.	c.c.		1	2	3	4	4½	5	5½	6
1.	5·0	·1	–	–	–	3·9	0	0	0	0	0	0	0	0
2.	2·5	·1	–	–	2·5	4·8	0	0	0	0	0	0	0	0
3.	5·0	·01	–	–	–	5·2	0	0	0	0	0	0	0	0
4.	–	–	–	–	5·0	5·35	0	0	1	1	2	4	4–5	5
5.	–	–	2·5	·01	2·5	5·45	0	1	1–2	2–3	4	5	5–6	6
6.	–	–	1·0	·1	4·0	5·6	0	0	1	1–2	2	3	3	4
7.	–	–	3·0	·1	2·0	6·0	0	1	1	2	3	3	3–4	5
8.	–	–	4·0	·1	1·0	8·2	0	1	1–2	2	3	4	4–5	5
9.	–	–	5·0	·1	–	8·4	0	1	1–2	3	3–4	4	4–5	5–6
10.	–	–	1·0	1·0	4·0	10·0	0	0	0	0	0	0	0	0

TABLE VIII.

pH Optimum for the Protease.

1% enzyme extract in sea-water, 5 c.c. Acid or alkali and/or water to 5 c.c. Blood fibrin, 0·2 gm. Incubated for 5 days. Temperature, 30° C.

No.	Acid		Alkali		H_2O	pH	NaOH ·01N
	c.c.	N.	c.c.	N.	c.c.		c.c.
1.	5·0	·01	–	–	–	3·4	1·25
2.	3·0	·01	–	–	2·0	3·75	6·9
3.	2·0	·01	–	–	3·0	4·0	0·6
4.	1·5	·01	–	–	3·5	4·85	2·05
5.	1·0	·01	–	–	4·0	5·3	8·4
6.	·75	·01	–	–	4·25	5·65	11·3
7.	·5	·01	–	–	4·5	6·0	32·5
8.	–	–	–	–	5·0	6·4	56·6
9.	–	–	·5	·01	4·5	6·6	8·5
10.	–	–	·75	·01	4·25	6·8	16·15
11.	–	–	1·0	·01	4·0	7·4	22·6
12.	–	–	1·25	·01	3·75	8·2	11·25
13.	–	–	1·5	·01	3·5	8·4	75·0
14.	–	–	5·0	·01	–	9·4	16·4

In order to determine whether the two optima were present when a polypeptide was used as a substrate the following experiment was carried out.

A 10% solution of peptone was used as the substrate and the optimum range of activity determined for the production of amino acids. Table IX and Text-Figure 12 show the result of this experiment; it will be noticed that while the range of activity extends from pH 5·6 to pH 8·8 there is now only one optimum point situated at pH 6·8, the higher optimum at pH 8·4 having been eliminated.

TABLE IX.

pH Optimum for the Protease.

1% enzyme extract in sea-water, 5 c.c. Acid or alkali and/or water to 5 c.c. 10% solution of peptone, 3 c.c. Incubated for 22 hours. Temperature, 29°–30° C.

No.	Acid		Alkali		H_2O	pH	NaOH ·01N
	c.c.	N.	c.c.	N.	c.c.		c.c.
1.	5·0	·01	–	–	–	5·1	45·8
2.	–	–	–	–	5·0	5·35	50·0
3.	–	–	5·0	·01	–	5·6	50·0
4.	–	–	1·0	·1	4·0	6·05	43·8
5.	–	–	2·0	·1	3·0	6·45	69·6
6.	–	–	3·0	·1	2·0	6·8	69·7
7.	–	–	4·0	·1	1·0	7·25	62·7
8.	–	–	4·5	·1	·5	7·6	63·3
9.	–	–	5·0	·1	–	7·9	63·2
10.	–	–	·4	1·0	4·6	8·4	59·6
11.	–	–	·5	1·0	4·5	8·8	58·2
12.	–	–	·6	1·0	4·4	9·15	57·1

In view of the fact that the pH of the gut lies between 5·8 and 6·7, the higher optimum cannot be of use to the animal during digestion. It is probable, however, that it may be an autolytic enzyme such as Shinoda (1928) found in Astacus, and this supposition is strengthened by the absence of this optimum when peptone was used as a substrate. Shinoda also found differences between the optimum pH for the working of the protease on different substrates such as have been recorded above.

6. ABSORPTION.

(*a*) Introductory.

This subject has been one of great controversy amongst the workers on the physiology of digestion in Isopods for many years. An excellent résumé of the work of previous authors is provided by Nusbaum-Hilarowicz (1917) in a preliminary summary of his work, and at greater length in the introduction to his paper of 1920.

As mentioned above in the description of the intestine, McMurrich (1896) showed that the so-called midgut is lined throughout by chitin and thus, according to him, cannot absorb, but can serve only for the storage of food, whereas Murlin showed this cuticle to be porous and demonstrated the absorption of fat and certain albuminous substances. This latter author did not discuss the question of absorption in the hepato-pancreas, though he dealt with the structure of its epithelium and with its function of secretion.

(*b*) Methods and Results.

In the investigation of the absorption of food in Ligia, the method used was that frequently employed by workers of recent years. Iron, in the colloidal form of ferrum lacticum or ferrum oxydatum saccharatum, in this case the latter, was fed to the animals under investigation. After a given period the gut and hepato-pancreas were dissected out and fixed, the usual fixative employed being a 5% solution of ammonium sulphide in 95% alcohol. In this case Yonge's modification (1926b, p. 709) of mixing the ammonium sulphide in alcohol with an equal volume of Bouin's fixative, just before use, was employed with satisfactory results. The sections were treated for 10 minutes with a 10% aqueous solution of potassium ferrocyanide, followed by a few minutes in a dilute solution of HCl, a bright Prussian blue colouration resulting wherever the colloidal iron had been absorbed. The sections were counter-stained with alum carmine.

By means of this treatment it was possible to demonstrate absorption throughout the whole length of the epithelium of the intestine and also in that of the hepato-pancreas, as shown in Plate I, Figures 1–5.

In the latter organ the granules found usually at the bases of the cells, termed zymogen granules by many authors, appear to act as centres round which the absorbed material aggregates.

In the experience of most workers who have employed this technique to demonstrate absorption, the colouration due to the iron tends to be concentrated in localised areas, somewhat as in Figures 4 and 5 which illustrate absorption in the hepato-pancreas. In the intestine the absorbed material was distributed much more diffusely, dense in patches as shown in Figures 1–3, but the greater mass of absorbed material (not illustrated) appeared a much paler blue with denser centres, gradually fading away towards the periphery of each region of absorption.

This effect was met with by Steudel (1912) when investigating absorption in the insects and is well illustrated by him. He describes the appearance in the following words : " Unterhalb des Kernes erscheint vielfach das Plasma selbst (ob nur scheinbar oder in Wirklichkeit, ist zweifelhaft) diffus blau gefärbt. Im diffusen Plasma liegen die Eisenkörnchen in allen

Grössen, teils deutliche Flecken, teils Punkte, teils kaum als solche zu unterscheidende feinste Pünktchen, die einen allmählichen Ubergang zu der erwähnten diffus bläulichen Grundfärbung bilden."

It is interesting to note that, though the chitinous lining of the intestine in Ligia is not perforated, yet absorption occurs very largely in this region of the alimentary canal. This can best be seen in Figures 2 and 3, Plate I, in which the absorbed iron is shown in actual transit through the chitinous layer.

One is struck by the resemblance between the relationship of this layer to the underlying epithelium and that of the peritrophic membrane to the absorptive epithelium in insects.

Steudel considers that the peritrophic membrane should be regarded as an organ of protection and compares it thus with the filter of Astacus. He states, moreover, that it allows only dissolved food to come into contact with the epithelium. The chitinous membrane in Ligia appears to function in an exactly similar way and to be analogous to the peritrophic membrane of the insects.

One further point of interest might be noted. McMurrich (1896, p. 93) remarks on the vacuoles "appearing as more or less extensive blisters of the epithelium" which he noticed on the inner surface of the epithelium of the "midgut" in the Isopods he examined. This peculiar condition was met with to a very great extent in Ligia after feeding, suggesting that it is associated with the absorptive processes. Its true significance is yet to be discovered.

7. SUMMARY.

1. Ligia is an omnivore, though normally feeding on Fucus. Portions of food are cut off by the mandibles and passed into the œsophagus. Its feeding period is limited.

2. The foregut is provided with a number of ampullæ and lamellæ which are furnished with spines and bristles and are so arranged as to form a filter for the separation of the liquid portion of the food from the solid particles.

3. A second filter is found in the floor of the foregut protecting the entrance to the hepato-pancreas.

4. A ventral valve is present preventing the entry into the hepato-pancreas of solid food from the intestine.

5. The hepato-pancreas consists of three pairs of tubules provided with a muscular network for producing contractions. The epithelium possesses discrete cells.

6. The intestine extends from the point of entry of the hepato-pancreas

into the foregut, to the rectum. Its epithelium is a syncytium lined throughout by a homogeneous layer of chitin. No typhlosole is present.

7. The rectal epithelium is syncytial, chitin lined and muscular.

8. Food entering the foregut is subjected to pressure which forces liquid through a filter, whence it passes into the hepato-pancreas.

9. The solid food is passed back, mixed with secretion from the hepato-pancreas and further pressed to expel liquid which passes through a second filter into the hepato-pancreas.

10. This is further aided by the tubules of the hepato-pancreas which force the secretion into the lumen of the gut and withdraw liquid by rhythmical contraction and relaxation.

11. The functioning of the foregut and associated organs appears to be designed for quick extraction of nutriment from the food.

12. The pH of the foregut averages 6·3 ; that of the hepato-pancreas, 6·0 ; of the intestine, 6·5 ; and of the rectum, 6·2.

13. The digestive enzymes are secreted by the hepato-pancreas ; none is secreted by the intestine.

14. The enzymes act readily on most carbohydrates, proteins and fats.

15. No cellulase is present, nor any enzyme which will act on pentosans.

16. The range of activity of the sucroclastic enzymes lies between pH 5 and pH 7 ; the optimum occurs between pH 5·65 and pH 5·8.

17. The proteoclastic enzymes are more powerful than the sucroclastic.

18. The range of activity for the liquefaction of gelatine lies between pH 5·35 and pH 8·5, with optima about pH 5·45 and pH 8·4.

19. Digestion of blood fibrin proceeds most rapidly at pH 6·4 and pH 8·4.

20. The range of activity for the digestion of peptone lies between pH 5·6 and pH 8·8 ; the optimum condition of pH being at 6·4. No second optimum was found.

21. The existence of an optimum at pH 8·4 probably indicates the presence of an autolytic enzyme.

22. Absorption was demonstrated by feeding with ferrum oxydatum saccharatum, and occurs in the epithelium of both the hepato-pancreas and intestine.

23. The appearance of the absorbed iron in the intestine was diffuse and resembled that described by Steudel in insects.

24. An analogy is suggested to exist between the chitinous membrane lining the epithelium of the intestine of Ligia and the peritrophic membrane lining the same region in the insects.

I wish to thank Dr. C. M. Yonge for his help throughout the course of this investigation and for reading the manuscript before publication.

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EXPLANATION OF PLATE.

FIG. 1.—Portion of syncytial epithelium of anterior region of intestine in longitudinal section, showing region of absorption demonstrated by iron saccharate. 24 hours after feeding. × 400.

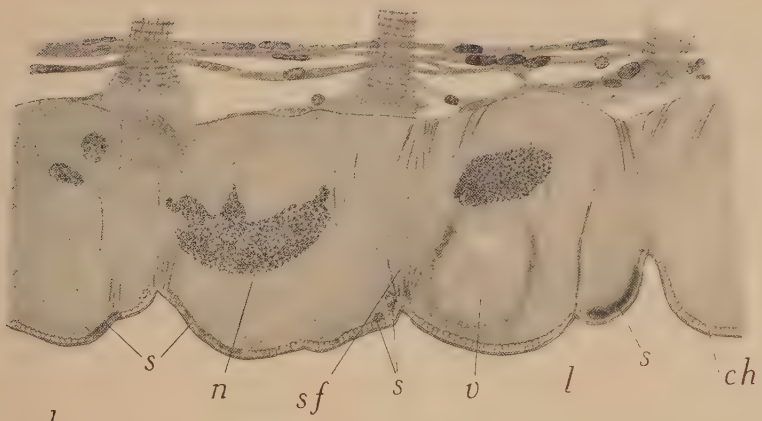
FIGS. 2 AND 3.—Portions of Fig. 1 enlarged to show absorption of the saccharate through the chitin. × 815.

FIG. 4.—T.S. through hepato-pancreatic tubule, showing absorption of iron saccharate. × 100.

FIG. 5.—Cell from epithelium of hepato-pancreas, demonstrating absorption of saccharate. × 425.

ABBREVIATIONS USED IN PLATE.

ch., chitin; *l.*, lumen; *n.*, nucleus; *nl.*, nucleolus; *s.*, iron saccharate; *s.f.*, "supportive fibres"; *v.*, vacuole.



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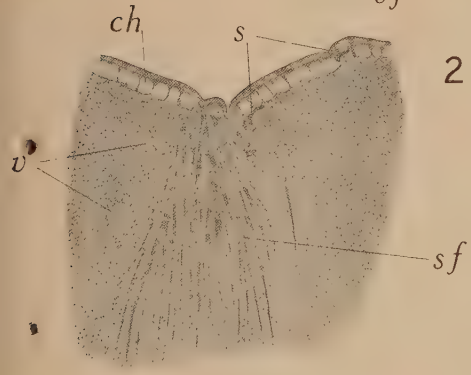

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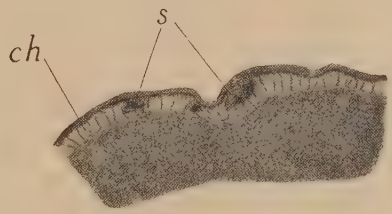

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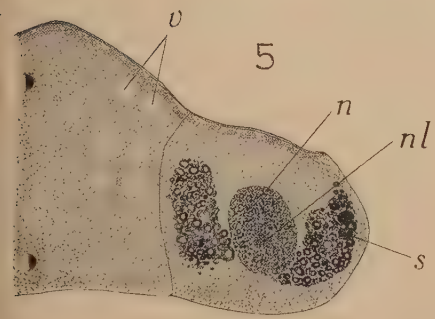

5

A Study of the Respiration and of the Function of Hæmoglobin in *Planorbis corneus* and *Arenicola marina.*

By

Mabel A. Borden, M.A.

This work was carried out while holding an 1851 Exhibition Scholarship.

With 15 Figures in the Text.

CONTENTS.

PAGE

INTRODUCTION 709

EXPERIMENTAL METHODS AND RESULTS

A. *Planorbis corneus*

1. Normal Rate of Respiration 710
2. Blood Volume 711
3. Oxygen Capacity of the Blood 713
4. Spectroscopic Examination of the Hæmoglobin
   (*a*) Under Anaerobiosis. 714
   (*b*) Under Aerobiosis following Anaerobiosis 717
5. Oxygen Debt Experiments 719
6. Discussion 722

B. *Arenicola marina*

1. Normal Rate of Respiration 724
2. Blood Volume 725
3. Oxygen Capacity of the Blood 726
4. Spectroscopic Examination of the Hæmoglobin
   (*a*) Under Anaerobiosis 726
   (*b*) Under Aerobiosis following Anaerobiosis 727
5. Oxygen Debt Experiments 728
6. Habitat and Habits 731
7. Discussion 735

SUMMARY 735

REFERENCES 737

INTRODUCTION.

THE presence of hæmoglobin in the invertebrates has generally been found to be correlated with a habitat at times deficient in oxygen; the function assigned to the hæmoglobin being either that of storing oxygen, or of transporting it at low pressures. Thus Leitch (1916) has shown that in Planorbis the hæmoglobin is used only when the oxygen pressure is so low that the necessary amount cannot be supplied by diffusion. Barcroft and Barcroft (1924) suggested that in Arenicola it acts "as a reserve store which the organism can use up when it has not access to sea-water." This

indicates that in Planorbis and Arenicola, both of which have hæmoglobin dissolved in the plasma and are adapted to environments in which the oxygen concentration must at times be considerably reduced, the function of the hæmoglobin is different.

Hill (1913–24), Meyerhof (1920) and others have shown that the fundamental mechanism of muscular contraction is an anaerobic one, by which nearly the whole of the energy is liberated in the breakdown of carbohydrate, as glycogen, to lactic acid ; while the recovery process, consisting of the restoration of a portion of the lactic acid to glycogen, is aerobic. It has also been proved that when an isolated muscle is stimulated in an oxygen-free atmosphere lactic acid accumulates, and the tissue goes into debt for oxygen, the magnitude of the debt being proportional to the quantity of the acid formed.

Slater and Davis (1926–28) have demonstrated that this phenomenon occurs also in the whole animal. They found that the common cockroach and the earthworm, when subjected to oxygen lack, went into debt and by this means were able to survive short periods of anaerobiosis.

It appears, therefore, that there are two mechanisms by which an animal can obtain the oxygen necessary for its life. Firstly, the circulatory system functioning under normal conditions ; secondly, the initial phase of muscular metabolism providing an emergency mechanism by which the animal is tided over short critical periods of oxygen deficiency.

In view of these facts it seemed of interest to study the respiration of, and the function of hæmoglobin in, Planorbis and Arenicola, and to find more exactly the significance of the hæmoglobin. Is it for the latter a storer of oxygen and for the former a transporter at low pressures, or are the animals able to go into oxygen debt and, therefore, not dependent on their blood for a supply of oxygen during anaerobiosis ?

EXPERIMENTAL METHODS AND RESULTS.

A. *PLANORBIS CORNEUS.*

1. Normal Rate of Respiration.

The respiratory rate was measured by observing the oxygen intake in Barcroft's differential blood gas apparatus (1914). Small snails weighing about 0·5 gm. were used, one animal being used for each experiment. The snail, having been first lightly dried to remove moisture and organic matter from the shell, was weighed and placed in the right-hand flask of the apparatus. About 2 c.c. of water were added to each flask, a slight additional amount being put in the left-hand flask to compensate for the volume of the snail. In the reservoir of each was placed a small piece of caustic soda to absorb carbon dioxide. The flasks were then attached to the U-shaped manometer and the apparatus set shaking in a water-bath,

the temperature of which closely approximated that of the room and was constant to within 0·5° of 15° C. The oxygen intake was recorded every 15 minutes for several hours, ample time having been first allowed for conditions of equilibrium to be established within the apparatus.

The average oxygen intake for a number of animals was 0·026 c.c. per gm. per hour. The results from which this figure was obtained are expressed in Table I.

TABLE I.

No. of Animal.	Weight gm.	Oxygen consumption in c.c. per hour. 1	2	3	4	5	6	Average in c.c. per gm. per hr.
1.	0·49	0·022	0·015	0·021	0·017	0·024	0·019	0·040
2.	0·62	0·016	0·014	0·014	0·012	0·018	—	0·024
	0·65	0·017	0·021	0·014	0·017	0·013	0·016	0·025
	0·66	0·017	0·017	0·010	0·018	0·015	0·017	0·024
3.	0·68	0·020	0·015	0·020	0·022	0·020	0·020	0·029
	0·69	0·028	0·024	0·027	0·027	0·020	0·022	0·036
	0·71	0·013	0·011	0·016	0·012	0·020	0·014	0·020
4.	0·68	0·017	0·013	0·014	0·015	0·013	0·012	0·021
5.	0·70	0·012	0·011	0·013	0·013	—	—	0·018

It may be seen from the above Table that the oxygen consumption of a snail weighing 0·49 gm. was 0·040 c.c. per gm. per hour, while that of snails weighing on the average 0·67 gm. was 0·024 c.c. per gm. per hour, or 0·016 c.c. less than that of the smaller animal. It is thought that, as in the case of the cockroach (Davis and Slater, 1926), the apparent relationship between the body weight and oxygen consumption is in reality a correlation between age and oxygen intake, the younger animals having a quicker metabolic rate. The oxygen consumption of two snails, Nos. 2 and 3 respectively, was measured for each on three different occasions. The results for each are fairly uniform.

Figure 1 represents the oxygen intake measured at 15° C. over a period of six hours, for a snail weighing 0·64 gm.

2. The Blood Volume.

Estimations of the blood volume were made in a Duboscq colorimeter. The snail was bled as thoroughly as possible, the blood being collected from the pulmonary cavity into which it had been driven by an artificially stimulated contraction of the foot. About 0·5 c.c. was thus obtained.

A known volume of blood was diluted to 10 c.c. with distilled water and used as the standard solution in the colorimeter. The rest of the snail was chopped up under a known volume of water to extract the blood from its tissues, and the wash-water was then filtered from the macerated

tissue by suction through a Buchner filter. Ten cubic centimetres of the diluted blood were then matched against the standard solution in the colorimeter, and the concentration of hæmoglobin in the sample of wash-water estimated according to the formula $C_1 = \frac{R_2}{R_1} \times C_2$, where R_1 and R_2 are the readings on the colorimeter of the unknown and standard solutions; C_1 and C_2 their respective concentrations. Care was taken always

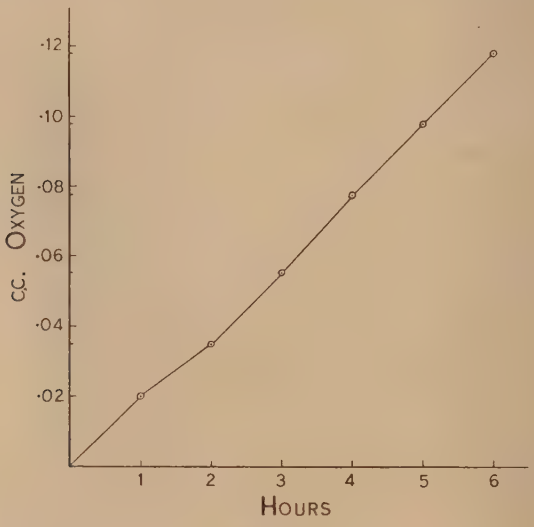

Fig. 1.

to use the same calibrated pipettes for measuring the solutions. The data of one estimation are tabulated as follows:—

TABLE II.

Showing Estimation of Total Blood in One Snail.

Weight of snail 1·61 gm.
Undiluted blood 0·42 c.c. made up to 4·2% aqueous solution.
Wash-water 18·65 c.c.

Colorimetric Readings.

No.	R_2	R_1	C_2	C_1
1.	1·27	1·65	4·2	3·23
2.	1·13	1·48	4·2	3·21
3.	1·08	1·37	4·2	3·31
4.	1·22	1·60	4·2	3·20
5.	1·23	1·54	4·2	3·35

Mean Value for C_1, 3·26%, therefore in 18·65 c.c. wash-water there is 0·61 c.c. blood, and the total volume of blood in the snail is 1·03 c.c.

The results of the experiments in Table III show the average blood volume to be 0·58 c.c. per gm.

TABLE III.

No.	Weight of Snail in grams.	Volume of Blood in c.c.	Volume of Blood per gm. of Snail.
1.	0·75	0·47	0·63
2.	1·10	0·74	0·67
3.	1·14	0·86	0·75
4.	1·44	1·00	0·69
5.	1·54	0·79	0·40
6.	1·56	0·98	0·63
7.	1·58	0·68	0·43
8.	1·61	1·03	0·65
9.	1·83	0·83	0·45
10.	1·90	0·98	0·51

3. The Oxygen Capacity of the Blood.

The oxygen capacity of the hæmoglobin was estimated by the ferricyanide method in Barcroft's apparatus and at a later date additional experiments were made in Van Slyke's manometric gas apparatus.

The experimental procedure was similar to that employed by Barcroft (1914), and Van Slyke and Neill (1924) respectively. A slight modification of the Barcroft apparatus was, however, introduced by using a small glass tube, as suggested by Keilin (1928, p. 217), to hold the ferricyanide solution in place of the reservoir in each flask more generally used for that purpose. The tube was hooked on to the reservoir in such a manner that the contents, and if desirable the tube itself, could be upset into the flask. This proved a more convenient method of bringing the ferricyanide in contact with the blood as, owing to the small amount of reagent used, great difficulty was formerly experienced in overcoming the effect of surface tension in the narrow reservoir.

The snails were bled as described above, undiluted blood from several individuals being mixed and well shaken to saturate the blood with oxygen. One cubic centimetre of the mixture was used for each measurement of the oxygen capacity.

The experiments showed that in 1 c.c. of blood the amount of oxygen held by the hæmoglobin was approximately 0·013 c.c., the total oxygen content of the blood 0·014 c.c. The results from which these values were obtained are given in Table IV.

TABLE IV.

Estimations made on 1 c.c. of Blood.

	Combined Oxygen by Barcroft's Apparatus in c.c.	Total Oxygen Capacity of Blood by Van Slyke's Apparatus in c.c.
	0·011	0·015
	0·010	0·014
	0·013	0·014
	0·011	—
	0·015	—
	0·016	—
Mean	0·013	0·014

The length of time that the total oxygen content of the blood will last the animal was calculated as follows :—

Blood volume per gm. of snail	0·581 c.c.
Oxygen content per c.c. blood	0·014 c.c.
Oxygen capacity of blood per gm. of snail	0·0081 c.c.
Oxygen consumption per gm. per hour	0·026 c.c.

Hence oxygen content of blood will last the animal 18 minutes.

4. Spectroscopic Examination of the Hæmoglobin.

(*a*) *Under Anaerobiosis*

Although the oxygen reserve of the blood and the extent to which it serves as a store of oxygen had been estimated, it seemed desirable to make a direct observation of the blood pigment during anaerobiosis. Stated briefly, the method of obtaining the information consisted of placing a number of animals in an inert gas ; obtaining and examining a sample of blood at frequent intervals ; noting at what time the bands of oxyhæmoglobin were replaced by that of reduced hæmoglobin. The examination of the blood was in every instance made under anaerobic conditions.

The apparatus for examining the blood was comprised of a microspectroscope fitted to a compound microscope. This, with the animals, was housed in a large box into which a steady flow of nitrogen could be maintained. Figure 2 illustrates the general structure of the box, the more particular features of which, however, require a brief description.

The back and upper half of the front were of glass, the rest of the box being made of wood, one inch in thickness. The top was removable to allow for the entrance of the microscope which, when once in place, was not withdrawn until the conclusion of the investigation. The top could be screwed down firmly and the junction between it and the rest of the box

was made air-tight by means of putty. To allow for focussing the microscope, the draw-tube projected through an opening in the top, the opening being lined with baize, so that the tube fitted snugly and yet could be moved up or down. At the bottom right-hand side was situated an inlet tube for nitrogen, the outlet being located on the opposite side near the top. Near the inlet tube a small door was cut, and by means of this opening the animals were admitted to and withdrawn from the box. The aperture into which the door fitted was lined with baize, and during the experiments when the door was shut the outside was covered with adhesive tape to prevent the leakage of nitrogen through the cracks, the pressure of nitrogen inside the box tending to be greater than atmospheric. In the lower half of the front two fairly large holes were made and over each the wrist part of a rubber glove was stretched and held in place by being firmly nailed, between two strips of elastic, to the wood. Seccotine was then run round the outside edge of the join which proved to be air-tight. By means of the gloves it was possible, without letting in any air, to put one's hands inside the box and thus manipulate the animals. Both the inner and the outer surfaces of the box were painted, the inside also being coated with paraffin wax.*

The nitrogen used for producing an oxygen-free atmosphere was filtered on its way to the box through alkaline hydrosulphite and alkaline solutions of pyrogallol to remove the traces of oxygen usually present in the commercially prepared gas. At the commencement of each experiment the air inside the box was swept out with nitrogen for half an hour or longer, the outlet tube was then closed, the door opened and the animals quickly placed inside. A constant flow of nitrogen was maintained throughout the experiment, the excess escaping around the draw-tube of the microscope. The atmosphere in the box was frequently tested for oxygen by Haldane's gas analysis apparatus. A connection between the latter and the outlet tube had been established with pressure tubing. Tests showed that the atmosphere in the box was never entirely anaerobic, the partial pressure of oxygen being approximately 0·5 mm.

At frequent intervals separate snails were bled directly on to a clean glass slide, and the blood examined for the presence of reduced hæmoglobin. The whole operation of bleeding the snail and noting the condition of the hæmoglobin took only a few seconds.

On account of the ability of Planorbis to hold a quantity of air in its lung it was necessary, before placing the snails in the nitrogen-box, to expose them for a few minutes to a vacuum. The investigation of the time taken for the blood to be reduced was also performed on snails in

* I am indebted to the Department of Botany, University College, London, for the use of the microspectroscope and to Mr. A. G. Nicholls for the construction of the nitrogen-box.

which the air had not been removed from the lung. Comparison of the results of these two types of experiments revealed the extent to which the lung supplemented the blood as a store for oxygen.

The results are given in Figure 3.

A survey of the investigation shows that for the experiments in which the snails were without air in their lungs the hæmoglobin was fully reduced, in some instances after subjection to only 10 minutes anaerobiosis, and in all cases some reduction had occurred; while after 25 minutes it was,

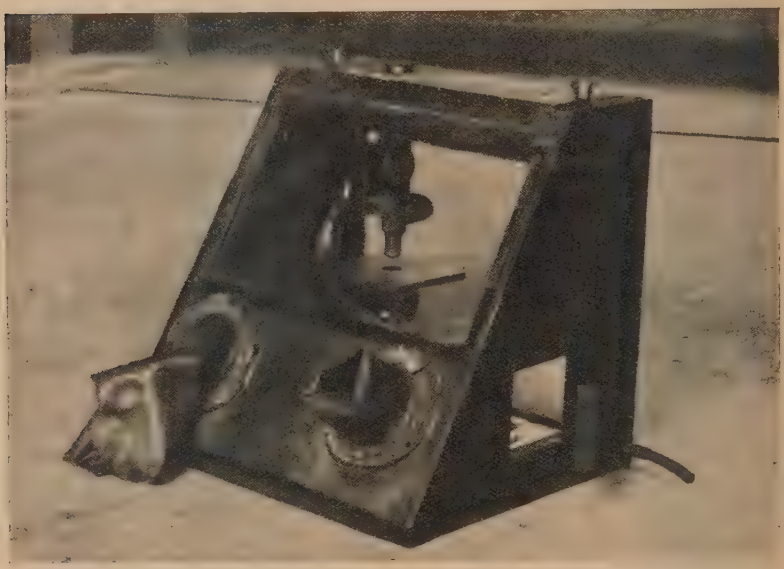

Fig. 2.

with two exceptions, fully reduced. If, however, the snails were used with their lungs full of air, the blood remained in the form of oxyhæmoglobin for 40 minutes, while between 40 and 80 minutes both the oxy- and reduced forms of hæmoglobin were found. After 80 minutes it was, with a few exceptions, completely reduced.

It is, therefore, clear that the total oxygen supply in the blood will last from approximately 20 to 25 minutes, and this is in fairly close agreement with the theoretically determined value of 18 minutes. The air in the lung will supply oxygen for 40 minutes, so that altogether Planorbis is well able to endure an hour's subjection to oxygen deficiency.

The experiments also substantiate Leitch's evidence that the combined oxygen is the last to be used by the animal but, whereas she found only

enough for three minutes and did not think it constituted a reserve, this investigation indicates that it may indeed be a reserve of some importance, since the oxygen supplied by the blood considerably prolonged the animal's survival in an anaerobic atmosphere.

(*b*) *Under Aerobiosis following Anaerobiosis.*

It was important with reference to subsequent oxygen debt experiments to ascertain if the animals, having been exposed to an anaerobic atmosphere, oxidised their hæmoglobin immediately on admittance to air. If

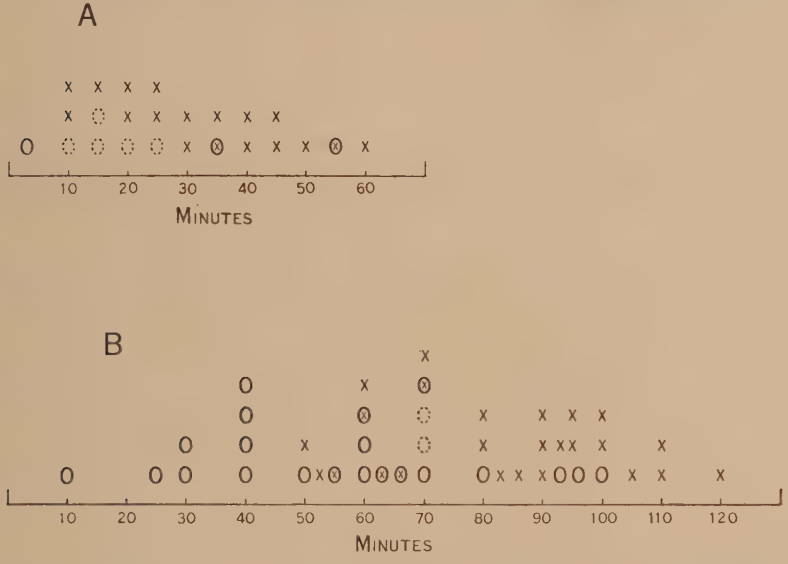

FIG. 3.—Diagram illustrating the time taken for the reduction of oxyhæmoglobin in Planorbis; A, in snails in which air had been removed from lungs; B, in snails with air in lungs. Separate animals were examined at frequent intervals. O, indicates presence of oxyhæmoglobin; ◌, presence of partially reduced hæmoglobin; ⊗, presence of almost completely reduced hæmoglobin; X, presence of reduced hæmoglobin.

N.B. The condition designated as partially reduced hæmoglobin was that in which the bands of oxyhæmoglobin were very faint; that of almost completely reduced hæmoglobin in which the faint oxyhæmoglobin bands were almost entirely masked by a weak reduced hæmoglobin band.

oxidation were not almost instantaneous the amount thus used would be included in the oxygen intake, thus giving a false value to the oxygen debt.

These experiments were, therefore, undertaken to measure the time taken by the animals for oxidising their hæmoglobin. For this purpose it was again necessary to carry out in the nitrogen-box the procedure of

exposing the animals to oxygen deficiency until the blood was reduced. They were then admitted to air in groups of two for varying periods, the blood being, however, collected and examined under anaerobic conditions. An additional piece of apparatus, a small stoppered bottle, placed on the floor of the nitrogen-box, was so arranged by means of an inlet tube that it could be connected directly with either the nitrogen supply or compressed air, while an outlet tube, extending to the exterior of the box, provided an escape for whatever gas was run into the bottle.

The experimental technique was as follows : nitrogen having been run into the box and into the bottle sufficiently to expel the air, the outlet tubes were closed and the animals placed in the box, the flow of nitrogen being continuous.

The blood from several snails was inspected until the hæmoglobin was found to be reduced ; two were then exposed to a current of air in the bottle for a definite time, at the end of which they were removed and the blood examined. Before removing the snails, however, the air in the bottle was expelled by a flow of nitrogen so that there was no introduction of air into the nitrogen-box. The details of a typical experiment are given in Table V, and the collective results in Figure 4.

TABLE V.

R indicates reduced hæmoglobin ; O, oxyhæmoglobin.

No.	Period of Anaerobiosis in min.	Time in Air in min.	Condition of Hæmoglobin.
1.	60	0	R
2.	62	0	R
3.	65	2	R
4.	65	2	R
5.	90	5	R
6.	90	5	R
7.	105	10	R
8.	105	10	R
9.	135	30	O
10.	135	30	O

During the above experiment the partial pressure of oxygen in the box was estimated after 45, 75, and 120 minutes, the values obtained being respectively 0·60, 0·70, and 0·53 mm.

From an examination of Figure 4 it will be seen that Planorbis does not oxidise its hæmoglobin immediately on exposure to air. Oxidation starts after 10 minutes and is complete within 20 minutes.

5. Oxygen Debt Experiments.

This investigation was undertaken to find if Planorbis went into oxygen debt when the period of anaerobiosis exceeded that provided for by its reserve of oxygen. The evidence so far obtained indicated that the reserve was sufficient for approximately one hour.

The presence of an oxygen debt following an anaerobic period would be marked by an increase in the oxygen consumption equivalent to the oxygen used by the animal at rest during the same period in air.

The method of measuring the oxygen debt in these experiments was as follows: one snail was weighed and placed in the Barcroft apparatus with

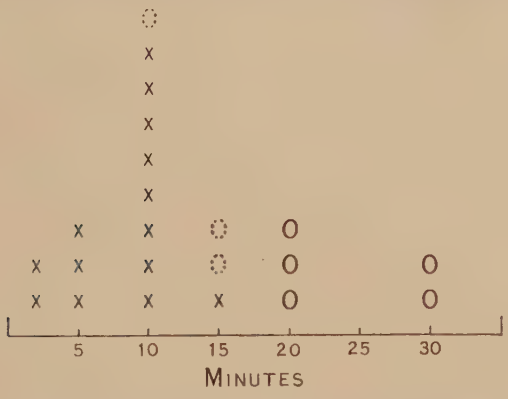

Fig. 4.—Diagram illustrating the time taken for oxidation of the hæmoglobin in separate animals (Planorbis). O, represents the presence of oxyhæmoglobin; ◌, the presence of partially oxidised hæmoglobin; X, the presence of reduced hæmoglobin.

one cubic centimetre of water and some caustic soda to absorb carbon dioxide. The apparatus was kept shaking in a water-bath, at 16° C. Observations were made on the animal's oxygen intake over a period of two to three hours, then by means of pressure tubing connecting the apparatus with the nitrogen supply, suction pump and pressure gauge the apparatus was emptied of air and refilled several times with nitrogen, the snail being left in this anaerobic atmosphere for whatever length of time was required. At the completion of the anaerobic period the apparatus was refilled with air and the oxygen intake again noted for several hours. Commercial nitrogen, before being introduced into the apparatus, was filtered through alkaline hydrosulphite and washed in several alkaline solutions of pyrogallol. Although these precautions were taken to purify the gas a trace of oxygen, amounting to a partial pressure of about 0·5 mm.,

was always present. This did not, however, appear to upset the experiments.

It is important to remember when interpreting the results, that the observed increase in respiration will be due in part to the oxidation of the hæmoglobin and that this amount must, therefore, be subtracted before the true value of the oxygen debt can be ascertained.

Since the average blood volume was estimated at 0·58 c.c. per gm. of snail; the oxygen capacity of the hæmoglobin at 0·013 c.c. per c.c. of

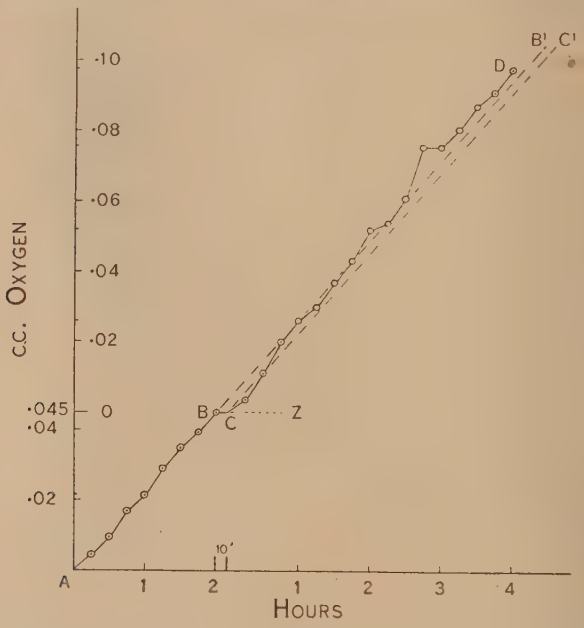

Fig. 5.—Graph showing the oxygen consumption of a snail weighing 0·48 gm. The period of experimental anaerobiosis was 1 hour; the period of true anaerobiosis, 10 minutes; temperature 16·5° C.

blood, it follows that the combined oxygen of the hæmoglobin, 0·0075 c.c. per gm. of snail, will be the amount needed for the oxidation of the hæmoglobin. It was also necessary to calculate the time the combined oxygen would last the animal, this being done for each on the basis of the normal oxygen intake during the experiment. This amount, plus 40 minutes for the supply of oxygen in the lung, was then deducted from the period of experimental anaerobiosis. Thus, if a snail were subjected to two hours oxygen deficiency and the hæmoglobin estimated to have enough oxygen for 20 minutes, the period of true anaerobiosis would be one hour; and from the observed oxygen intake following anaerobiosis was subtracted the

amount needed for oxidation of the hæmoglobin. These corrections were applied to all experiments.

Figures 5, 6, 7, and 8 illustrate typical experiments. The line AB shows the normal oxygen intake before anaerobiosis; BZ the period of experimental anaerobiosis; BC the period of true anaerobiosis estimated by deducting from BZ the length of time for which the oxygen in the lung

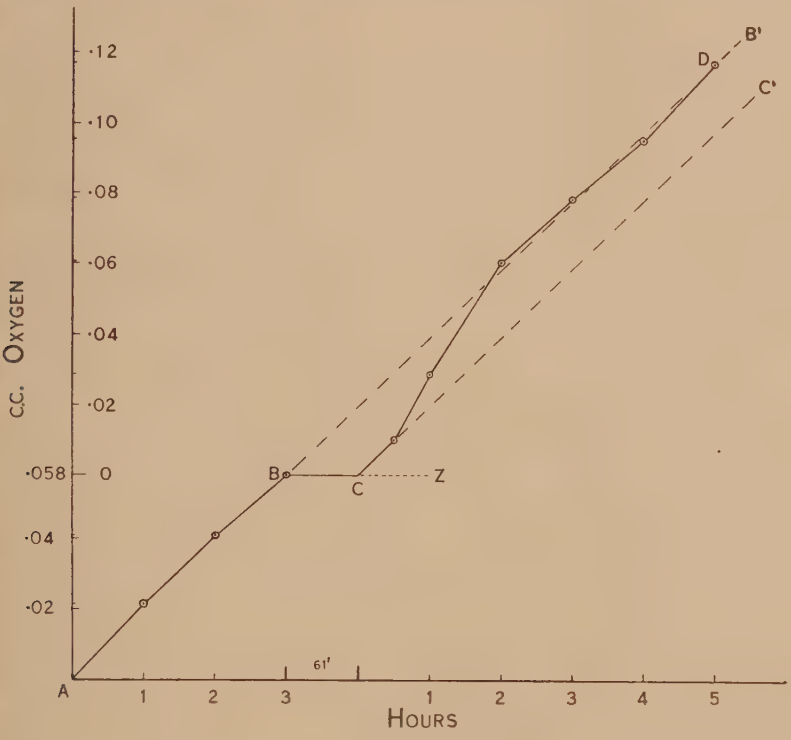

Fig. 6.—Graph showing the oxygen consumption of a snail weighing 0·82 gm. The period of experimental anaerobiosis was 2 hours; the period of true anaerobiosis, 61 minutes; temperature 15° C.

and that combined in the blood would last the animal. The broken line BB′ represents the amount of oxygen it is assumed the animal would have used had it not been subjected to anaerobiosis; the broken line CC′ is that along which post-anaerobic respiration would have proceeded had it been equal to the normal resting value. The line CD shows the oxygen consumption after anaerobiosis less the estimated amount needed for oxidation of the hæmoglobin.

It will be seen from Figures 5, 6, and 7 that when the anaerobic period

has ended, the animal takes up more oxygen than it normally requires, the excess being equal to the amount it would have used during the time it was in nitrogen. Figure 8 illustrates an experiment in which the period of anaerobiosis did not extend beyond the time for which the animal was provided with oxygen by its lung and there was, therefore, no debt.

The results show that for short anaerobic periods up to approximately one hour, the extent to which anaerobiosis was carried, Planorbis went

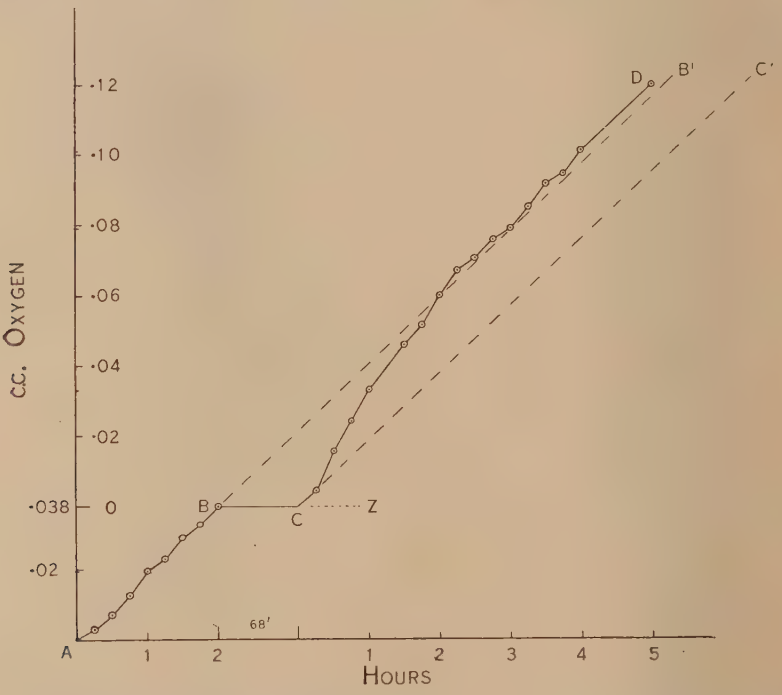

Fig. 7.—Graph showing the oxygen consumption of a snail weighing 0·50 gm. The period of experimental anaerobiosis was 2 hours; the period of true anaerobiosis, 68 minutes; temperature 16° C.

into debt for oxygen. The magnitude of the debt was proportional, within the limits of experimental error, to the time of anaerobiosis; the period of recovery was longer.

6. Discussion.

Planorbis inhabits rivers, canals, ponds and marshes, the water of which may be deficient in oxygen during periods of drought and stagnation. It has been shown by Leitch (1916) that normally enough oxygen is supplied to the snail by diffusion through the surfaces exposed to the

water. When the oxygen pressure falls below that necessary for diffusion the hæmoglobin is reduced and, to facilitate the oxidation of the blood, the animal renews the air in its lung by rising frequently to the surface. The present investigation shows that the combined oxygen is sufficient

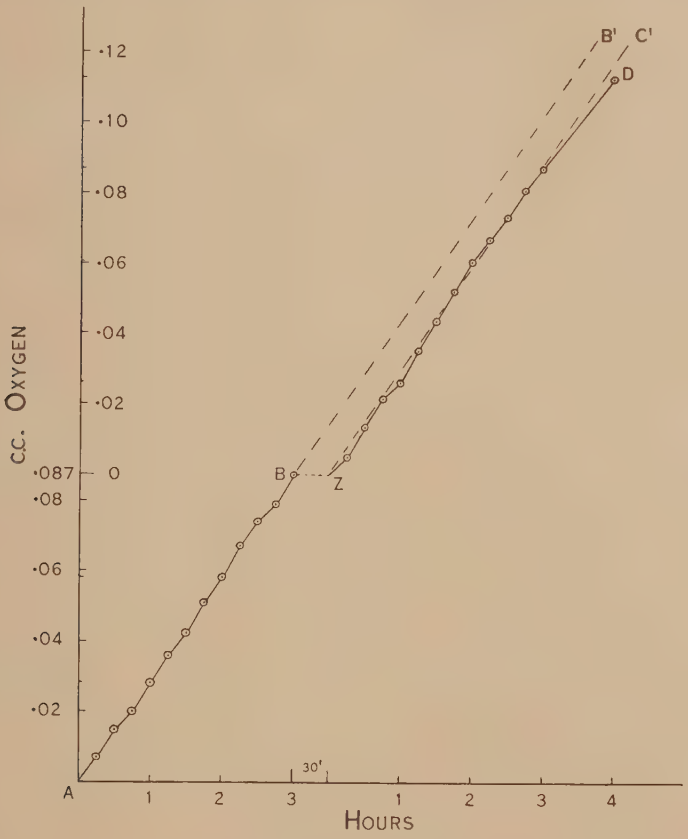

FIG. 8.—Graph showing the oxygen consumption of a snail weighing 0·50 gm. The period of experimental anaerobiosis was 30 minutes. There was no period of true anaerobiosis. Temperature 16° C.

for approximately 25 minutes. Leitch's evidence that the combined oxygen is the last to be used by the animal is further substantiated.

Barcroft (1928) has shown that the main dissociation of Planorbis blood occurs between oxygen pressures of 1 to 10 mm. of Hg.

It is concluded that the hæmoglobin functions primarily as a transporter of oxygen at low pressures. The combined oxygen may be considered as a reserve only in the sense that it supplements the oxygen held

in the lung, with the result that the animal is for a time independent of reduced oxygen pressures in the water. The significance of the hæmoglobin is, therefore, that it furnishes the means whereby oxygen is secured and transported to the tissues at times of lowered oxygen pressure.

The importance of the lung should not be overlooked as, in addition to facilitating the oxidation of the blood, it provides a mechanism by which the animal can obtain oxygen as long as it has access to air.

The investigation establishes the ability of Planorbis to put up an oxygen debt. Planorbis appears, therefore, to be especially well adapted for survival in an environment at times deficient in oxygen.

B. *ARENICOLA MARINA.*

1. Normal Rate of Respiration.

The experimental procedure for the estimation of the oxygen consumption of Arenicola differed only slightly in detail from that already described for Planorbis. Measurements were carried out in Haldane's blood gas apparatus which was kept shaking in a water-bath, the temperature of which ranged between 10° and 12° C., the variation of the temperature for each experiment not exceeding 0·5° C. Carbon dioxide was absorbed by small strips of filter paper moistened with 10% sodium hydroxide solution. The measurements were made on worms varying in weight from 2 to 9 gm., one worm being used at a time and readings taken over a period of six hours. Except for rhythmic contractions of the body wall the worm remained quite still.

The results of six determinations are given in Table VI and show that the average oxygen consumption is of the order of 0·031 c.c. per gm. per hour. The large animals appear to use less oxygen than the small, but yet adult, animals. It seems, therefore, that in the case of Arenicola there may be a relationship between body weight and oxygen intake.

TABLE VI.

No. of Animal.	Weight in gms.	Oxygen Consumption in c.c. per hour. 1	2	3	4	5	6	Average, c.c. per gm. per hr.
1.	2·4	0·080	0·070	0·085	0·100	0·097	0·098	0·037
2.	3·6	0·170	0·150	0·150	0·152	0·118	0·132	0·040
3.	4·3	0·130	0·136	0·140	0·100	0·114	0·115	0·028
4.	5·2	0·125	0·100	0·120	0·090	0·115	0·090	0·020
5.	7·1	0·260	0·240	0·206	0·250	0·220	0·220	0·034
6.	9·5	0·315	0·273	0·252	0·265	0·255	0·245	0·028

In Figure 9 the general character of the results is exemplified by the curve which represents the oxygen used per hour at 11·6° C, by a worm weighing 5·2 gm.

2. Blood Volume.

The blood volume of Arenicola was determined by the same colorimetric method as that outlined for Planorbis. The manner of bleeding the worm was as follows : the worm was opened along the mid-dorsal line to expose the body-cavity : the cœlomic fluid allowed to drain out and the cavity dried as thoroughly as possible : the heart and blood vessels were punctured and the escaping blood removed from the body-cavity with a pipette. In this way it was possible to obtain about 0·5 c.c., the rest of

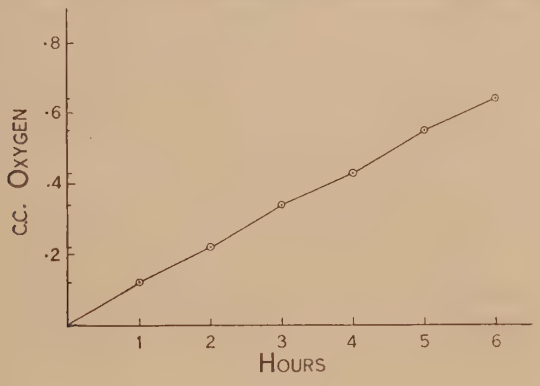

Fig. 9.

the blood being extracted by suction through a Buchner filter after the chopped-up worm had been left standing for some time under water.

In Table VII are given the results from which the average blood volume was estimated to be 0·382 c.c. per gm.

TABLE VII.

No.	Weight of Worm gm.	Volume of Blood c.c.	Volume of Blood per gm. of Worm.
1.	6·65	2·57	0·386
2.	6·87	3·43	0·500
3.	7·49	2·33	0·312
4.	7·70	2·56	0·333

It cannot be claimed that the colorimetric method is absolutely accurate and these results should be considered to be only roughly approximate to the true value. The presence of the black pigment from the epidermis rendered accurate colour comparison difficult.

3. Oxygen Capacity of the Blood.

Estimations of the total oxygen content of Arenicola blood were carried out in the Barcroft and in the Van Slyke apparatus, the procedure being identical with that already described for the experiments on Planorbis blood.

The results given in Table VIII show the oxygen capacity of the hæmoglobin to be 0·087 c.c. per c.c. of blood, the total oxygen content 0·097 c.c. per c.c. of blood.

TABLE VIII.

Estimations made on 1 c.c. of Blood at N.T.P.

Combined Oxygen by Barcroft's Apparatus c.c.	Total Oxygen Capacity of Blood by Van Slyke's Apparatus c.c.
0·097	0·098
0·085	0·097
0·084	0·097
0·085	0·096
—	0·099
—	0·096
Mean 0·087	0·097

The length of time that the total oxygen content of the blood will last the animal may be calculated as follows :—

Blood volume per gm. of worm	0·382 c.c.
Oxygen content per c.c. of blood	0·097 c.c.
Oxygen capacity of blood per gm. of worm	0·037 c.c.
Oxygen consumption per gm. per hour	0·031 c.c.

Hence, oxygen content of blood will last the animal 71 minutes.

4. Spectroscopic Examination of the Hæmoglobin.

(*a*) *Under Anaerobiosis.*

Arenicola blood was examined for the same purpose and by the same method as described for Planorbis. The worms were dried lightly on filter paper before being placed in the nitrogen-box. The process of obtaining the blood was as before, the worms being opened longitudinally, the blood vessels cut and a drop of blood transferred from the body-cavity to a glass slide and covered with a cover slip. The results are expressed in Figure 10.

The first sign of reduction occurred after 20 minutes, while after half an hour the hæmoglobin, although not completely reduced, was as nearly reduced as it was ever found. The analysis of the nitrogen atmosphere in the box showed a partial pressure of oxygen of about 0·5 mm., which

may perhaps explain the failure of the hæmoglobin to appear completely reduced. The dissociation curve of Arenicola hæmoglobin, as given by Barcroft and Barcroft (1924), showed that at this pressure the oxygen saturation of the blood is about 8%.

Since the hæmoglobin was never found in a state of complete reduction

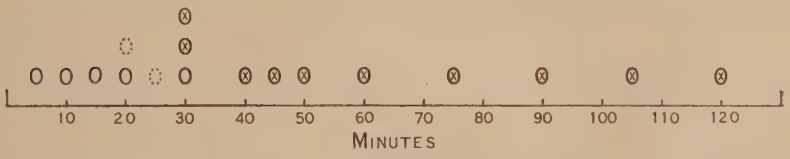

FIG. 10.—Diagram representing the time taken for the reduction of oxyhæmoglobin in Arenicola. Separate animals were examined at frequent intervals. O, indicates presence of oxyhæmoglobin; ◌, presence of partially reduced hæmoglobin; ⊗, presence of almost completely reduced hæmoglobin.

it is impossible to arrive at any further conclusion concerning the time the oxygen supplied by the blood will last the animal. This investigation shows, however, that the supply is sufficient for at least 30 minutes.

(*b*) *Under Aerobiosis following Anaerobiosis.*

Figure 11 shows the results obtained for Arenicola, the methods being the same as those used for Planorbis and described above.

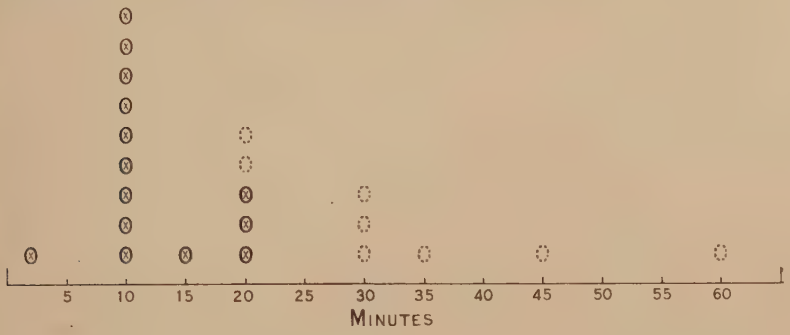

FIG. 11.—Diagram representing the time taken for the oxidation of the hæmoglobin in Arenicola. Separate animals examined at frequent intervals. ◌, indicates that some oxidation has occurred though it is not complete; ⊗, presence of almost completely reduced hæmoglobin.

It is interesting to note that the hæmoglobin was still in the reduced state after 10 minutes exposure to air, while after 20 minutes, although some oxidation had occurred, well defined bands of oxyhæmoglobin were never found. The explanation for this may be that the temperature at which the experiments were conducted, 20° to 22° C., was higher than that of the

animal's normal environment at that time of the year, which was approximately 15°C. This, coupled with a fairly long exposure to almost complete anaerobiosis, injured the animals to such an extent that recovery did not take place during the time of the experiment.

5. Oxygen Debt Experiments.

Two series of experiments on the ability of Arenicola to put up an oxygen debt were made in the Barcroft apparatus. In the first, the weighed worm was placed in the respiration flask of the apparatus with 1 c.c. of sea-water, and a strip of filter paper moistened with 10% solution of NaOH, to absorb carbon dioxide, was placed in the reservoir, the anaerobic atmosphere being produced as before with nitrogen.

In the second series, hydrogen, washed through sodium hydrosulphite, pyrogallol and silver nitrate solutions, replaced nitrogen. No water was added, the flasks being simply rinsed with sea-water and used moist. Carbon dioxide was absorbed by a small rectangle of filter paper wet with 10% solution of KOH.

The measurement of the oxygen debt was made by observing the normal oxygen intake immediately before and after the period of oxygen lack, anaerobic conditions within the apparatus being attained as before. Analysis of the gas, whether nitrogen or hydrogen, revealed a partial pressure of oxygen of about 0·5 mm.

Observations made during the period of anaerobiosis showed that the volume of gas in the respiration flask gradually diminished, constant volume being attained after about 30 minutes, from which it was concluded that the worm used this small amount of oxygen and was not, therefore, under strictly anaerobic conditions until no more oxygen was available. In a few experiments constant volume was never attained and these were discarded. It was noted that the worm was capable of movement at the completion of the anaerobic period.

To estimate the period of true anaerobiosis allowance was made for the time taken by the worm to use the oxygen impurity in the apparatus and the combined oxygen in its blood. A mean value for the combined oxygen was obtained as follows :—

Volume of blood per gm. of worm	0·382 c.c.
Amount of oxygen combined with Hb per c.c. of blood	0·087 c.c.
Hence, amount of combined oxygen per gm. of worm	0·033 c.c.

The length of time that the combined oxygen will last is dependent on the animal's normal oxygen consumption.

To represent the true value of the debt a deduction was made from the increased oxygen intake following anaerobiosis corresponding to the amount needed by the animal for oxidation of its hæmoglobin. These

corrections were, at the best, only approximate, as they did not allow for individual variation in blood volume and concentration of hæmoglobin in the blood, nor for the fact that before constant volume was attained in the apparatus the worm must have been subjected to partial anaerobiosis.

Some typical results of the oxygen debt experiments are illustrated

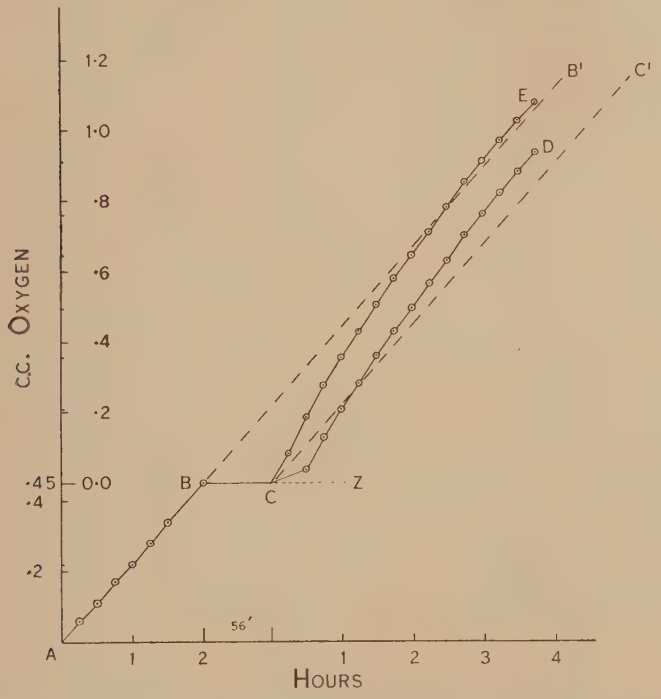

FIG. 12.—Graph showing the oxygen consumption of a worm weighing 4·5 gm. The period of experimental anaerobiosis in hydrogen was ·2 hours; the period of true anaerobiosis, 56 minutes; temperature 13° C.

by Figures 12, 13, 14, and 15 in which AB represents the normal oxygen intake measured before anaerobiosis; BZ the period of experimental anaerobiosis; BC the period of true anaerobiosis estimated by deducting from BZ the length of time needed for constant volume to be attained within the apparatus and that for which the combined oxygen of the blood was calculated to last the animal. The broken line BB′ represents the oxygen it is assumed the animal would have used had it not been deprived of air; the broken line CC′ the oxygen intake equal to the normal resting value. The line CD shows the oxygen intake following anaerobiosis, the

correction for the oxidation of hæmoglobin having been applied; while CE is the total oxygen intake following anaerobiosis as measured during the experiment.

Figures 12 and 13 illustrate experiments in which a partial debt was found. It will be noticed that the excess oxygen intake was equal to about half what the animal would have used during the same time in air. Figure 14 is typical of the results of the majority of experiments in which there appeared to be no oxygen debt. Figure 15 illustrates an experiment in which the period of experimental anaerobiosis was 30 minutes, the

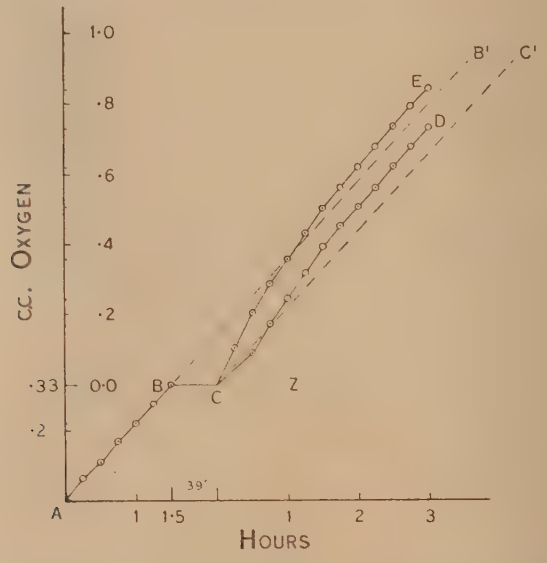

Fig. 13.—Graph showing the oxygen consumption of a worm weighing 3·4 gm. The period of experimental anaerobiosis in hydrogen was 1 hour 36 minutes; the period of true anaerobiosis, 39 minutes; temperature 14° C.

oxygen impurity in the apparatus enough for 3 minutes and the combined oxygen in the blood sufficient for 40 minutes. The worm was, therefore, not under anaerobic conditions as far as its blood was concerned, and thus there should be no debt, as was found to be the case when the amount needed for the oxidation of the hæmoglobin was subtracted from the observed oxygen intake.

The investigation on the ability of Arenicola to go into oxygen debt has given, on the whole, inconclusive results. The first series of experiments, in which nitrogen was used, indicate that the oxygen consumption after anaerobiosis was below normal. The second series of ten experiments

with hydrogen showed two instances of a partial oxygen debt. If, however, no deduction for the oxidation of the hæmoglobin is made, two out of the six experiments in which nitrogen was used show an oxygen debt, the remaining four a partial debt. Five of the experiments with hydrogen give a debt and four others only a partial oxygen debt.

It seems reasonable to conclude that Arenicola will oxidise its hæmoglobin in the course of one or two hours. Although the correction allowed for this may be too great, as is indicated by the experiments in which the

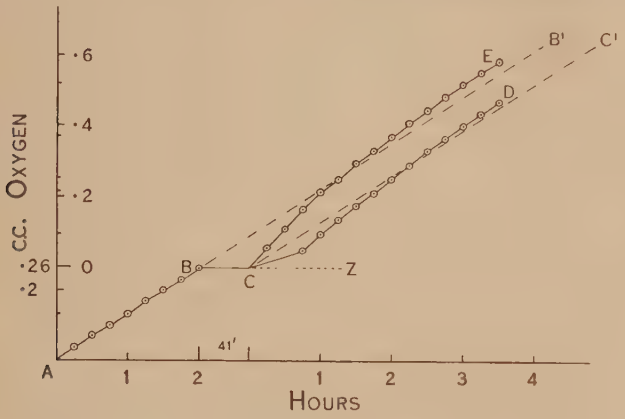

FIG. 14.—Graph showing the oxygen consumption of a worm weighing 3·6 gm. The period of experimental anaerobiosis in hydrogen was 2 hours; the period of true anærobiosis, 41 minutes; temperature 13° C.

corrected value for the oxygen intake falls below normal, the failure of the majority to show an oxygen debt cannot be entirely due to the error introduced by this correction.

6. THE HABITAT AND HABITS OF ARENICOLA.

The worms used for this research were collected from a sandy beach forming part of Batten Bay, on the east side of Plymouth Sound. The area inhabited by Arenicola is uncovered between high and low tides for approximately three hours. The sand is black, with the exception of the surface layer, which is brown to a depth of one-quarter to one-half an inch. It is well known that black sand indicates the presence of sulphides from decomposing organic matter, while the brown colouration is the result of the oxidation of sulphides by the atmosphere. Arenicola burrows to a depth of one to two feet below the surface. The burrow, which is U-shaped and open at each end, is constructed by the animal pushing and eating its way through the sand. The entrance is not sealed at low tide by the castings. These are, in general, coiled in a heap around the opening in

such a manner that the latter is clearly visible. It was observed that whereas the surrounding sand is black that lining the burrows is brown, similar to the surface sand. The brown layer is approximately 1 mm. in thickness and extends throughout the entire length of the burrow.

The oxygen content of the interstitial water was determined as follows. Samples of the water from one foot below the surface were taken from a marked area on three occasions, each sample consisting of four lots of water collected at intervals of approximately one hour, so that the first was obtained just as the tide uncovered the area and the fourth just as the tide again reached the area. The boring apparatus used to obtain the

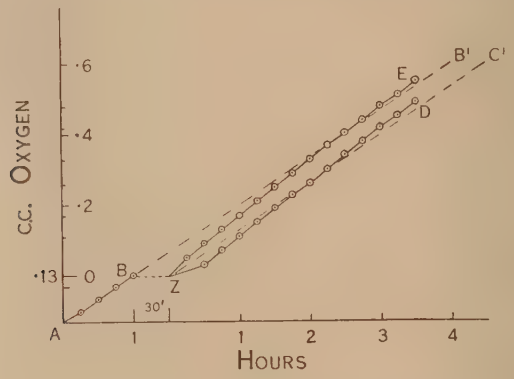

Fig. 15.—Graph showing the oxygen consumption of a worm weighing 2·8 gm. The period of experimental anaerobiosis in nitrogen was 30 minutes. There was no period of true anaerobiosis as the worm had a supply of oxygen sufficient for 43 minutes. Temperature 11° C.

samples consisted of a hollow iron tube, two inches in diameter and two and a half feet in length, closed at the bottom and terminating in a spiral spike. Near the bottom was a double row of holes, 5 mm. in diameter, blocked on the inside by means of a plunger which could be withdrawn as desired. The borer was forced into the sand to the required depth and the plunger then removed so that the water could drain in through the holes. The water was obtained by sucking it up into a large separating funnel the stem of which reached to the bottom of the borer. Three successive samples of water were thus collected and run into bottles of approximately 250 c.c. capacity. Precautions were taken to keep the water as free as possible from contact with air by sampling under liquid paraffin.

The estimation of oxygen was made in the laboratory by Winkler's method. Time was first allowed for the sediment invariably drawn up

with the water to settle. This was necessary as the sediment contained sulphides which on the addition of acid liberated hydrogen sulphide, the latter combining with the iodine from Winkler's reagents. The bottles were then opened and water free from sediment, drawn off from the upper layer, was transferred under oil to smaller bottles of 60 c.c. capacity. The estimations of dissolved oxygen were then carried out.

The remaining water in the large bottles was tested for hydrogen sulphide by adding acid to samples of water free from sediment and to other samples well mixed with sediment, and testing these with lead acetate paper. It was found that the water which had been shaken up with sediment generally gave a positive test for hydrogen sulphide, whereas that lacking sediment invariably gave a negative test. It was, therefore, concluded that the method of allowing sediment to settle before estimating the oxygen was sufficient to prevent the interference of hydrogen sulphide with Winkler's reagents.

Control experiments were devised to find the margin of error introduced into the oxygen estimations by the unavoidable handling of the water in the field. For this purpose sea-water was boiled to expel air and samples siphoned into several of the 60 c.c. bottles. The rest of the boiled water was then sucked up into the separating funnel, run into 250 c.c. bottles, and samples transferred from them to other 60 c.c. bottles under oil. The technique was as far as possible an exact reproduction of that employed in the sampling of water from the field. The oxygen concentration was determined by Winkler's method. The difference between that found in the siphoned samples and that in which the water was transferred by the separating funnel represents the error introduced by the handling of the water. The average error introduced by this method of sampling the water was found to be 0·44 c.c. of oxygen per litre. Table IX gives the oxygen content in c.c. per litre of the interstitial water.

TABLE IX.

Date of Sampling.	Oxygen content of water from sand in c.c. per litre at Exposure.	1 hour.	2 hours.	3 hours.
Oct. 24.	0·107	0·058	0·142	0·145
	0·066	0·151	0·137	0·034
	0·114	0·125	0·165	0·028
Nov. 7.	0·137	0·021	0·136	0·130
	0·126	0·055	0·124	0·197
	0·114	0·073	0·241	—
Nov. 10.	0·212	0·220	0·130	0·266
	0·199	0·135	0·151	0·125
	0·218	0·117	0·206	0·143

The highest value of oxygen found was 0·27 c.c. per litre, and as the experimental error was 0·44 c.c. per litre it is clear that there is practically no oxygen in the interstitial water at any time. The variation in the values of oxygen present in samples collected at a given time may, perhaps, be explained by the amounts of sediment in the bottles being different, and by the difference in the length of time the water was exposed to the sediment.

The data so far presented show that the worms burrow in black sand, the interstitial water of which at a depth of 1 foot lacks oxygen. The burrows, however, are lined with brown sand, indicating that oxidation has occurred.

A few observations have been made in the laboratory on the burrowing habits of Arenicola. It was noticed that in the process of burrowing the worms acted as small suction pumps, that is, by everting and inverting the proboscis water was drawn towards the anterior end from all sides. The worms begin their burrows when covered with water and it is clear that as they move into the sand water will flow in behind them, thus oxidising the lining of the burrow which consists of particles of sand held together with mucus and forming a layer about 1 mm. in thickness. It was noted in one case where a worm had died in its burrow and lost blood that the red colouration did not diffuse into the surrounding water. This indicates that the water in the burrows cannot be considered as part of the interstitial water.

It is presumed that when the tide is high the worms have no difficulty in obtaining oxygen, as they can either maintain a current through the burrow or come into direct contact with fresh sea-water by moving to the surface.

During the period of intertidal exposure they are most generally found at a considerable depth below the surface. It is probable that during this period, which lasts for approximately three hours, conditions approaching anaerobiosis will occur. The water in a completed burrow was observed in the laboratory to move and to change its direction of motion suddenly, as the worm alternately protruded and withdrew its proboscis. Fine particles of sediment were observed in motion within the burrow. It is, therefore, assumed that the movement of the worm within its burrow will keep the water in a state of constant motion, thus bringing it all into contact with the air at the surface. Since the openings are not more than 0·5 cm. in diameter the surface of water exposed to the air is obviously restricted and thus only a limited amount of oxygen can be dissolved in a given time. This amount will, however, be used by the worm as the dissociation of its blood occurs mainly at very low oxygen pressures. It is thought that the oxygen thus acquired, in conjunction with that already combined with the hæmoglobin, will be sufficient to satisfy the animal's requirements during low tide.

7. Discussion.

Barcroft and Barcroft (1924) have shown that the dissociation of Arenicola blood takes place mainly at oxygen pressures of between 1 and 3 mm. of Hg. It is evident that in well aerated water respiration is effected by the diffusion of oxygen through the gill filaments into the blood stream, the transport of oxygen by the hæmoglobin taking place only at reduced pressures.

The great affinity of the hæmoglobin for oxygen and the consequent low pressure at which it dissociates adapt Arenicola particularly well to its environment.

It is concluded that the primary function of the hæmoglobin is that of transporting oxygen during the period of lowered oxygen pressures to which the worms are probably subjected at low tide. The theoretically calculated amount of combined oxygen is enough for an hour and, while it is not suggested that the hæmoglobin functions chiefly as a storer of oxygen, the reserve it holds will be of great service to the animal.

The experiments on the ability of Arenicola to go into oxygen debt gave on the whole unsatisfactory results. In a few instances the oxygen intake following anaerobiosis was in excess of the normal. This would seem to indicate that Arenicola is able to go into debt for oxygen, but that its ability to do so is limited. The limiting factors may perhaps be a low buffering power on the part of the tissues and blood. The concentration of lactic acid was not estimated. In view of the results obtained this will be necessary before it is possible to conclude to what extent the mechanism of the oxygen debt is of use to the animal.

SUMMARY.

A study of the respiration and of the function of hæmoglobin in *Planorbis corneus* and *Arenicola marina* has been undertaken.

The oxygen consumption of Planorbis is of the order of 0·026 c.c. per gm. per hour, measured at 15° C.

The blood volume is approximately 0·581 c.c. per gm.

The combined oxygen of the blood is 0·013 c.c. per c.c. of blood, the total oxygen being 0·014 c.c. per c.c. The total oxygen capacity of the blood per gm. of snail is estimated to be 0·0081 c.c.

The oxygen supplied by the blood is calculated to last 18 minutes and is found by experiment to last 25 minutes.

The snail does not immediately oxidise its hæmoglobin after subjection to anaerobiosis. Oxidation begins after 10 minutes and is complete within 20 minutes.

Planorbis goes into debt for oxygen when subjected to short anaerobic

periods. The debt is proportional to the time of anaerobiosis, but the recovery period is longer.

This animal appears to be well adapted for survival in a habitat which at times may be deficient in oxygen.

The function assigned to the hæmoglobin is that of transporting oxygen.

The oxygen consumption of Arenicola is of the order of 0·031 c.c. per gm. per hour, measured at between 10° and 12° C.

The blood volume is approximately 0·382 c.c. per gm.

The combined oxygen of the blood is 0·087 c.c. per c.c. of blood, the total oxygen content being 0·097 c.c. per c.c. The total oxygen capacity of the blood is estimated to be 0·037 c.c. per gm. of worm.

The oxygen supply of Arenicola blood is calculated to last 71 minutes and is found by experiment to last at least 30 minutes.

Arenicola when transferred from anaerobic to aerobic conditions partially oxidises its hæmoglobin after 20 minutes. Instances of complete oxidation were never found.

The results of the oxygen debt experiments are not conclusive. A few instances of a partial debt were found. It is thought that the ability of the worm to go into oxygen debt is limited.

The sand at Plymouth, in which the Arenicola burrow, is black, while that lining the burrows is brown, similar to the surface sand. The burrows open to the surface at each end and the openings are not blocked by the castings. The amount of oxygen in the interstitial water is found to be negligible at all times. The water in the burrows is not considered to form part of the interstitial water. It is thought that the movements of the worm within the burrow keep the water in motion so that oxygen is being continually renewed at the surface.

The dissociation of Arenicola hæmoglobin occurring at low oxygen pressures of between 1 and 3 mm. of Hg. seems especially adapted to the needs of the animals. The significance of the hæmoglobin is that it functions as a carrier of oxygen during the period of lowered oxygen pressures to which the animals are liable to be subjected during intertidal exposure.

Acknowledgments.

This work was carried out in the Department of Zoology, University College, London, and at the University's table at the Laboratory of the Marine Biological Association, Plymouth. I wish to thank Professor D. M. S. Watson, F.R.S., and Mr. C. F. A. Pantin, who suggested this piece of work, for their advice and criticism. I should also like to express my thanks to Dr. E. J. Allen, F.R.S., and the members of his staff for placing the facilities of the Laboratory at my disposal.

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Hydroid Pigments. I. General discussion and pigments of the Sertulariidæ.

By

Nellie M. Payne.

INTRODUCTION.

Cœlenterate Pigments.

The literature on cœlenterate pigments is not large, but is rather scattered and somewhat difficult of access. In addition some pigment studies on squids and worms appear in the literature as researches on cœlenterates. An effort has been made in this paper to bring together the known results that have been secured in the study of cœlenterate colouring matter with the exception of such papers as deal with protective coloration, mimicry, and the like. Compilations are not listed unless they contained an original contribution either in methodology or view-point. Only papers where it could be clearly ascertained that the animal studied was a cœlenterate have been cited. In some of the older work done by non-zoologists, the scientific name has been sunk to a mere synonym and the description of the animal was insufficient even to place it to phylum.

The pigments of the cœlenterates include several types of compounds, some of which may be used in respiration. Blanchard (1882) described a blue, water-soluble pigment obtained from the circum-umbrellar region of *Rhizostoma cuveri*. The aqueous solution of this pigment showed three absorption bands in the red, yellow, and green respectively. Colasanti (1880) described a blue pigment from hydromedusæ, which colouring matter he considered as a compound of carotin and protein. Elmhirst and Sharpe (1920) found a light-sensitive pigment in *Actinia equina* and *Anemonia sulcata*. The intensity of the colour was proportional to the intensity of the light to which the Actinians were exposed. Elmhirst studied the environment in relation to the colour, and Sharpe the chemical properties of the pigments themselves. Sharpe came to the conclusion that in *Actinia equina* the red and brown pigments may be the same basic substance, the exact colour of which is due to the alkalinity or acidity of the medium in which the pigments occurs. This animal used blue and possibly violet-light rays; the light-sensitive pigment which it contains has properties similar to those of chlorophyll and is used in

respiration. *Anemonia sulcata* contained no hæmoglobin derivatives. It did contain small green algæ along the inner border of the tentacles and in the mesenteries. These algæ contained a colouring matter very similar to the chlorophyll of green leaves.

Fewkes (1889) described a case of pigment discharge by a hydroid medusoid. Fürth (1903) in his monograph on the physiology of lower animals classifies the pigments of cœlenterates into seven groups: (1) the blue pigment of the medusoid umbrella, (2) pelageine, (3) the blue colour of the coral *Heliopora cærulea* and compounds related thereto, (4) the hæmatin series, (5) the red and purple pigments which include colouring matters such as actinochrome, purpuridin, and the floridines, (6) the lipochromes, and (7) the uradines, a sulphur-containing group, which are yellow in the live animal, but turn black on its death.

Fulton (1922) described a red pigment from *Actinia bermudensis*. The pigment is soluble in CS_2, methyl, ethyl, and amyl alcohol, petroleum ether, pyridine, and acetone. The pigment occurs in granules. Addition of HCl or valeric acid deepened the red colour, but alkali produced no change that was apparent. Geddes (1882) found that the "yellow cells" in cœlenterates were commensal organisms. These organisms, which were algæ, produced oxygen which was needed by the cœlenterate in its respiration. The chlorophyll-like pigment, found in the cœlenterate Anthea and in others, was thus due, not to the cœlenterate itself but to the alga. Griffiths and Platt (1895) obtained a violet pigment from the medusoid Pelagia, to which dyestuff they applied the name pelageine and ascribed the formula $C_{20}H_{17}NO_7$. Since the pigment was extracted in amorphous condition only, it seems scarcely possible that the formula suggested could be valid. Griffiths (1892) considered that the cœlenterate pigments had a respiratory function.

Haurowitz and Waelsch (1926) found a blue pigment in *Vellela spirans*, which on spectroscopic examination showed absorption in both red and violet, but no distinct bands. The pigment became reddish brown on drying, or with treatment with alcohol or toluol. Kropp (1931) extracted the colouring matter of *Vellela spirans* with water. The extract was opalescent with a reddish colour in reflected light. Near the neutral point it turned yellow, and successively pink and reddish as the solution became increasingly acid. An aqueous solution of the pigment showed diffuse absorption in the red, $\lambda = 655\ \mu\mu - 685\ \mu\mu$, and blue violet $425 - 475\ \mu\mu$, but no sharp bands.

The most comprehensive work that has been made on the cœlenterate pigments is that of Krukenberg (1880, 1882). He obtained a green pigment with a red fluorescence from *Anthea cereus*.* The alcoholic solution of this pigment varied from brown to green. The absorption spectrum

* = *Anemonia sulcata*.

of the alcoholic solution was figured. Addition of acid changed the green colour to blue and added a new band at the junction of yellow and green. This author pointed out the similarity between the green pigments of the cœlenterates and the chlorophyll in plants.

Lancaster (1873) examined the pigment found in the stalked protozöon, *Stentor cœruleus*, with a Sorby-Browning spectroscope. He found an absorption spectrum containing two bands, the darker in the red extending a little to the side of solar C line, and the second in the green. *Stentor mülleri* gave absorption spectra like those of Hydra and Spongilla. Since Stentor feed on Hydra, it served as a concentrating agent for the hydroid pigment. Liversidge (1898) found a blue nitrogen containing pigment in the coral *Heliopora cœrulea*. The pigment from "dead coral" dissolved readily in alcohol and acetic acid. Heating a concentrated residue of coral extract produced an odour similar to that of burned fish. Liversidge tested the solubility of the material in a large series of compounds both inorganic and organic. Acetic acid proved to be one of the best solvents. An alcoholic solution of coral contained more organic matter than an acetic acid solution. The pigment was insoluble in kerosene, the pure paraffines, in CS_2, CCl_4, or petroleum ether. Solutions were either blue or green, depending on the solvent used, e.g. a sodium acetate solution was green; a formic acid solution blue. The pigment did not appear to be either a natural indicator or a reducing agent.

M'Kendrick (1881) described pigments that occurred in small granules in several of the cœlenterates. He did not study the chemistry but mentioned a yellow pigment in Chrysaora, a blue one in Cyanea, and a pink in Aurelia. He gave simple methods for pigment extraction.

MacMunn (1885, 1890) found colouring matters in cœlenterates which resembled these of vertebrate blood. *Actinia mesembryanthemum** contained a pigment which could be transformed either into hæmochromogen or hæmatoporphorin. This pigment was not actinochrome, which dyestuff is widely distributed in the Actiniæ. In *Sargartia parasitica* occurred a colouring matter in both reduced and oxidised states. This pigment did not occur in any of the Actiniæ. A green pigment which gave all the reactions of biliveridin was found in the mesenteries of *Anthea mesentherium*. A yellow pigment which differed from chlorophyll occurred in the tentacles of *Anthea cereus*,† *Bunodes balii*, and *Sargartia bellis*. In these species were both luteins and a pigment that gave absorption bands in the red and violet part of the spectrum.

Merejowski (1881) described a carotin-like substance from cœlenterata which he designated as tétranérythine.

Mosely (1873) found a pigment, the so-called actiniochrome, in *Actinia mesembryanthemum*‡ which is a pale olive or merely a dirty white colour

* = *Actinia equina*. † = *Anemonia sulcata*. ‡ = *A. equina*.

in muddy water. This colouring matter also occurred in *Bunodes crassicornis* which is a transparent green. In some specimens of *B. crassicornis* the tips of the gonidial tubercules were bright red. Mosely determined the absorption band for the red pigment but lost the drawing. He found no similar colouring matter in *Actinia mesembryanthemum** or *Actinia rosea*. In 1877 Mosely found a hematine-like pigment in *Bunodes crassicornis*. Teissier (1925) described an interesting case of pigment development which paralleled the embryonic. This author believed that the appearance of pigment in the eggs of *Clava squamata* was due to the liberation of carotin from a compound of carotin and protein.

General Chemical Literature.

The nomenclature for the various pigments differs from author to author. In the foregoing literature summary, the original term used by the investigator in question has been quoted. Lypochromes, luteins, and carotin belong to the group of carotinoids as the term is defined by Palmer (1922). In this paper the nomenclature of Palmer will be followed. This worker summarised the preceding work on carotinoid pigments and has also made valuable contributions of his own to the study of chromatology. Schertz (1925) described an accurate method for obtaining crystalline carotin. He extracted the pigment with a highly purified ether kept over sodium. Crystalline carotin kept in an ice box oxidised very slowly and could be stored some months without deterioration. Wheldale (1916) classifies plant pigments into two general groups: the plastid pigments which include chlorophylls, xanthophylls, and the carotinoids, and the pigments distributed throughout the cell or the anthocyanins, and their derivatives, the flavones. The anthocyanins and flavones are characterised by their water solubility. In the plant kingdom there are two yellows, one type soluble in water, the other in fat solvents. Wheldale also pointed out that many white flowers contain a pigment made apparent in alkaline solution. The flavones are natural indicators, being white in neutral or acid solution, and yellow to orange in alkaline. The flavones are oxidation products of the anthocyanins. The flavone group forms characteristic salts with $FeSO_4$, $FeCl_2$, $FeCl_3$, and $Pb(COO)_2$.

The ultimate source of the coelenterate pigments may be the plant kingdom. Thus far, to the author's knowledge, there has been no clear example of either chlorophyll or carotin being a direct product of animal metabolism. Geddes (1882) and others have demonstrated the algal origin of cœlenterate chlorophyll. An interesting paper by Palmer and Knight (1924) describes the transfer of potato carotin into the blood of the

* = *A. equina*.

potato beetle, *Leptinotarsa decimlineata* Say, and from this insect to the predaceous plant bug, *Perillus bioculatus Fabricius*, in which the carotin was in part deposited in the exoskeleton. Thus far the origin of the cœlenterate pigments other than chlorophyll is unknown. It is altogether possible that both carotin and flavones are of algal origin.

Materials and Methods.

The hydroids studied by the present writer belonged to two distinct groups, one including those bearing carotinoid pigments, namely, the Antennulariidæ and Haleciidæ; the other including the Sertulariidæ which carried water-soluble yellow and brown pigments.

The characteristic of the colouring matters in these two groups, respectively, was so different that the same method of treatment of the hydroids previous to extraction could not be used. The caratinoid-bearing group, of which *Antennularia antennina*, *A. ramosa*, *Aglaophenia pluma*, *A. tubulifera*, and *Halecium halecinum* were studied, were washed in fresh water repeatedly before their pigments were extracted. The hydroids thus freed of debris were dried in an incubator to air-dry state and then extracted with CS_2, CCl_4, or ether. The pigments in each of the species studied was more soluble in CS_2 than in any other of the solvents used. Those used included CS_2, CCl_4, ether, absolute alcohol, pyridine, petroleum ether, and chloroform. Details of the methods will be given in a later paper.

The colouring matter of the Sertulariidæ with the exception of the brown of *Sertularia pumila* was so extremely soluble in fresh water that the hydroids could not be freed of the usual debris and dirt clinging to them by washing in fresh water. The most practical way of cleaning hydroid material in these groups was washing in sea-water. The long stems were then cut into small pieces and extraction made in either fresh or dried condition. Alcohol or distilled water was the solvent used. A higher yield of pigment was obtained from fresh than from dried material. The crude extracts were allowed to evaporate, and during the evaporation were stirred. Since the pigments were less soluble in cold than in warm water, the extracts were chilled with ice to promote crystallisation. Crystals were obtained with great difficulty. Acid solutions of pigments from *Sertularella gayi* Lamaroux and *S. polyzonias* Linnæus yielded a small crop of crystals.

Although crystals of the pigments themselves were difficult to procure, it was relatively easy to procure their salts in crystalline form. Lead and iron salts of the pigments in the Sertulariidæ studied resembled the flavone salts of the plant flavones.

The species studied were *Sertularia pumila* Linnæus, *Sertularia argentea*

Ellis and Solander, *Sertularella gayi* Lamaroux, *Sertularella polyzonias* Linnæus, and *Thuiaria articulata* Pallas.

Results Obtained.

The nature of the pigments in the Sertulariidæ had first to be studied from a purely negative view-point. They were not soluble in the fat solvents, CS_2, CCl_4, or petroleum ether, therefore they were not carotinoids. Tests for free sulphur were negative, although the hydroids turned black in death. The species *Sertularia argentea*, *Sertularella gayi*, *S. polyzonias*, and *Thuiaria articulata* blackened readily on exposure to air. The yellow pigments found did not reduce Fehling's solution, nor did they present the other characteristics of chrysomphanic acid. The pigment in the Sertulariidæ is not distributed evenly throughout the tissues but occurs in small patches. This is especially true of the yellow species. The brown species *Sertularia pumila* apparently has more nearly uniform distribution of colouring matter.

No pigment solutions blackened on exposure to air. The blackening of the hydroid might be due to some enzyme adsorbed on the tissues, and not dissolved with the pigment. It is also interesting to observe that the one intertidal species studied, namely, *Sertularia pumila*, did not blacken on exposure to air. The other species used are normally covered at all times by sea-water. The blackening may possibly be that of a pigment not soluble in the same solvents as are the flavone-like pigments.

The yellow colouring matter in *Sertularella gayi* Lamaroux and *Sertularella polyzonias* Linnæus were extremely soluble in distilled water. An animal immersed in either distilled or tap water began to lose colour from the instant it was placed in the solvent. In neutral or alkaline aqueous solutions the pigment was a lemon-yellow. The gonophores were especially rich in colouring matter. Extracts from fresh material often had a greenish yellow cast. Rhomboid pigment crystals were obtained from etherial solutions. By salt formation, type of crystal, or differential solubility tests, the pigments from these two species, *Sertularella polyzonias* and *S. gayi*, could not be separated. It is possible that these closely allied species bear a common pigment. A determination of the chemical constitution of the pigments in question is necessary before it can be said definitely whether there are one or two pigments involved.

Nearly allied to the colouring matter in the Sertularellas is the chromatophore group in the white or colourless species *Sertularia argentea* Ellis and Solander. This hydroid can be used as indicator for hydrogen ion concentration. It is colourless in neutral and acid solutions. The aqueous extract of the chromatophore is colourless at pH 7, yellow at pH 8, orange at pH 8·5, and finally brown in pH 10. Extracts of these chromatophore

Species.	Colour in Nature.	Solubility.			Colour of metallic salts.			Influence of pH on pigment colour.		
		Water.	Alcohol.	Ether.	$Pb(COO)_2$	$FeCl_3$	$FeSO_4$	Neutral pH 7.	Alkaline pH 8.	Acid pH 6·5.
Thuiaria articulata Pallas	orange-brown	readily	readily	moderately	blue	brown	greenish	brown	lemon-yellow	colourless
Sertularia argentea Ellis and Solander	white or colourless	readily	readily	slightly	blue	blue	greenish	colourless	yellow (at pH 8·5 orange)	colourless
Sertularella gayi Lamaroux	lemon-yellow	readily	readily	moderately	blue	greenish	blue	yellow	yellow	colourless
Sertularella polyzonias Linnæus	lemon-yellow	readily	readily	moderately	blue	greenish	blue	yellow	yellow	colourless
Sertularia pumila Linnæus	brown	moderately	readily	slightly	greenish	greenish	blue	brown	brown	yellow (at pH 4 white)

in ether or alcohol were also colourless. The presence of the pigment became apparent when KOH or NH_4OH was added to the solutions. The pigment or rather the chromatophore group was insoluble in CCl_4, CS_2, or petroleum ether, but was slightly soluble in ether. The presence of the pigment group in this colourless hydroid can be demonstrated by holding the hydroid over NH_4OH fumes. It was by this simple method that Wheldale (1916) demonstrated the flavone in the white snapdragon.

The pigment in the *Sertularia pumila* was distinctly less soluble in water than were the pigments from the other species. It was insoluble in CS_2, CCl_4, and petroleum ether, even after long standing in contact with these solvents. *Sertularia pumila* is also a natural indicator for pH, but its colour changes lie on the acid side of neutrality. Characteristic salts of the pigment were obtained with $Pb(COO)_2$, $FeCl_3$, $FeSO_4$.

The orange-brown species *Thuiaria articulata* Pallas contained a pigment, also a natural indicator, which was readily soluble in water. Crystals were obtained from slightly acid solutions. Addition of small quantities of HCl to water, alcohol or ether solutions made them turbid. From these turbid solutions rhomboid crystals were obtained. The pH of such solutions must be controlled, for at pH 6·5 the pigment was decolorised. Characteristic salts were formed with $Pb(COO)_2$, $FeCl_3$, and $FeSO_4$. In solubility, crystal type, and salt formation the pigment of *Thuiaria articulata* resembles that found in the Sertularellas.

The accompanying table gives the chief results obtained with the pigments of the Sertulariidæ. Each of the species studied contained a water-soluble colouring matter. The pigments were all natural indicators. In solubility, crystal type, and salt formation the colours from *Sertularella gayi*, *S. polyzonias*, *Sertularia argentea*, and *Thuiaria articulata* showed marked similarity. *Sertularia pumila* contained a pigment which possessed properties similar to the flavones, but was distinctly less water soluble than the others studied.

SUMMARY.

1. The yellow colours of the hydroids include at least two groups, the carotinoids found in the Antennulariidæ and Haliciidæ and the flavone-like pigments that occur in the Sertulariidæ. From the literature a third group, the uradines, may be added.

2. The flavone pigments and their relatives are all water soluble and are thus distinguished from the carotinoids which are not water soluble, but may be dissolved in the usual fat solvents.

3. In the Sertulariidæ studied, a graded series of chromatophore groups and pigments was found which ranged from the colourless species *Sertularia argentea* Ellis and Solander, through the yellow of *Sertularella gayi*

Lamaroux and *S. polyzonias* Linnæus, to the orange-brown of *Thuiaria articulata* Pallas. The brown of *Sertularia pumila* is probably also a flavone derivative.

4. The flavones and related pigments occur extensively in the plant kingdom. It is probable that the flavone-like pigments found in the Sertulariidæ are of plant origin.

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Muds of the Clyde Sea Area. II. Bacterial Content.

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With 2 Figures in the Text.

Introduction.

An important factor in the economy of the sea is the presence of bacteria, which are found not only in the sea-water itself, but in the mud and sand of the sea bottom. The parts of the sea which are most important biologically are (i.) the upper twenty metres of water in these latitudes, and (ii.) the uppermost layer of the sea floor in regions where the water is less than a few hundred metres deep. The first is of interest as the zone inhabited at the proper season by autotrophic organisms, chiefly phytoplankton. It is here that the products of photosynthesis are largely formed ; upon these heterotrophic life in the sea is ultimately dependent. Except in shallow water, there is insufficient light at the sea bottom for photosynthesis, and the organisms inhabiting the sea floor are of necessity heterotrophic. These organisms, among which are protozoa and the majority of bacteria, derive their foodstuffs directly or indirectly from the already organised proteins and carbohydrates of dead organic matter accumulated on the sea floor. The bacteria found in this zone include the following representatives of different physiological groups :—

(i.) Simple heterotrophs, which can utilise the proteins of dead organisms, and which liberate ammonia as waste metabolite.

(ii.) Nitrifying organisms of the two types, (*a*) those which oxidise ammonia to nitrite, and (*b*) those which oxidise nitrite to nitrate (**14**). These bacteria thus assist in the regeneration of nitrate from ammonia. This is important, because although ammonium-nitrogen can be utilised by some algæ and by higher plants, nitrate-nitrogen is in general more readily assimilated by autotrophic plants. Now the low concentration of nitrate is a limiting factor to plant growth in the sea, so it is desirable to know what bacterial changes in the sea may result in its formation.

(iii.) Denitrifying bacteria. These are simple heterotrophs with a special mechanism for reducing nitrate and nitrite to elementary nitrogen, the oxygen thus liberated being used for respiration (see p. 762).

(iv.) Nitrogen-fixing bacteria, which fix elementary nitrogen by synthesising nitrogenous compounds, possibly ammonia. These have been isolated from algal slime along the coast-line (**5**, **6**), but not from true bottom deposits.

(v.) Sulphur bacteria, which oxidise hydrogen sulphide to free sulphur (**16**).

The physiological importance of these and other groups will naturally depend upon their numbers, and accordingly the experimental work described below was undertaken (i.) to determine the approximate numbers of bacteria present in samples taken from different types of bottom deposits in the Clyde Sea Area, and (ii.) to make a preliminary investigation into the different types of micro-organisms found.

Historical.

The earliest record of the bacteria in marine mud is that of Russel (**13**), who noted that there were many more micro-organisms in the mud than in the supernatant water. Drew (**2**) found that the exceptionally high bacterial content of shallow coastal waters in the tropics was due to the fact that the mud flats below were unusually rich in bacteria. These appear to be the only general records of mud bacteria.

Other workers have studied special groups of organisms isolated from marine muds. Nitrifying bacteria of both types were isolated by Thomsen (**14**) at Kiel from bottom deposits close to the shore. According to Issatchenko (**4**), nitrate-forming bacteria are found in northern seas in bottom deposits, but their number depends on the nature of the sea bottom; he found that they were absent from black muds, present in clay deposits, and more abundant in calcareous sandy deposits.

Experimental.

(a) *The Area Investigated.*

The samples examined were all from the Clyde Sea Area, a brief description of which is given by Marshall and Orr (**12**); the stations used for mud sampling are described by Moore (**9**, **10**). The bottom deposits are chiefly muds, with some sands; these are classified into four types by Moore (**10**), and stations representative of each of these four types were selected for bacteriological analysis as under :—

(i.) *Loch-head Type :* Station 10, Loch Striven Head. Depth 24 m.

(ii.) *Mid-loch Type :* Station 11, Clapochlar. Depth 73 m.

(iii.) *Deep-water Type :* Station 7B, Garroch-Corrie. Depth 166 m.

(iv.) *Sandy Type :* Station 1B, Kames Bay. Depth 25 m.

(b) *Sampling.*

The samples were obtained by means of the apparatus described by Moore and Neill (**11**). The sampling tube is a cylinder which is sent down open at both ends ; thus the water through which it passes streams through the tube and may deposit bacteria on the inside of the glass tube. However, any error thus introduced is negligible, since (i.) it appears that there is practically no streaming of mud up the sides of the glass tube when the sample is pushed through, (ii.) the number of bacteria in the muds is very much higher than that in the water above, and (iii.) the sub-samples were taken from the centre of the core, which had not been in contact with the glass.

(c) *Laboratory Technique.*

The mud samples were transported ashore in the sampling tubes. They were taken below deck in order to keep them cool during the interval between sampling and examination, and they were kept in the dark by means of cardboard sheaths. The time interval between sampling and plating depended on the distance of the sampling station from the laboratory, and varied from half an hour to four hours. On arrival at the laboratory the cylindrical tube was marked off into centimetre lengths, and at selected intervals two sub-samples (*a*) and (*b*) were taken from the mud. One sub-sample was used for determination of the interstitial water, and the second was used for estimating the bacterial content.

This portion of the work was carried out in conjunction with Mr. Moore, and the author wishes to acknowledge his kindness in taking the samples, in dividing them into sub-samples, and in determining the amount of interstitial water present in each sub-sample.

Sub-sample (*a*) was placed in a tared watch glass and weighed ; it was then dried to constant weight at 100° C. The difference between the first and the final readings gave the total amount of contained water. From these the figures the amount of water contained in sub-sample (*b*) was calculated. Sub-sample (*b*) was placed in a tared sterile Erlenmeyer flask and weighed ; filtered sea-water, sterilised by autoclaving for 30 minutes at 30 lbs. pressure, was then added to make up a volume of 100 c.c. ; the pipettes used were sterilised by heating to 150° C. for 30 minutes. The whole was then shaken very thoroughly to disperse the constituent mud particles. In the first series of dilutions (Station 7B, 14/4/30 and Station 1B, 10/4/30), lead shot was used to facilitate the

breaking up of the mud. The use of lead shot was then discontinued, on account of the toxic action of metals on most bacteria. It was difficult to disperse the mud particles in sub-samples from the lower layers of mud, which contained less interstitial water.

The diluted sample was then plated out on agar and on gelatine according to the procedure previously described by the writer (**7**). Four agar cultures and four gelatine cultures were made, a known volume of the diluted sub-sample being added. In the first samples examined, the amounts of inoculum were 1·0 c.c., 1·0 c.c., 0·5 c.c., and 0·5 c.c., but it was found that the colonies which developed were too crowded for convenient counting, and in the later work the following amounts were used : 0·5 c.c., 0·5 c.c., 0·1 c.c., and 0·1 c.c.

The plates were incubated at room temperature (15° C.) for five days, and then the bacterial colonies visible to the naked eye were counted. Tables I–IV give the results of the counts ; from these the average number of bacteria per c.c. in each diluted sub-sample was calculated. From this average the number per gram of dry mud was calculated approximately to the nearest thousand.

In estimating the bacterial content of mud the following difficulties occur :—

(i.) The relative amounts of water and of solid matter in sub-samples from different depths in any one series vary very much. The bacteria are present both on the particles and in the interstitial water, but since the organic matter in the mud is the source of their food supply, they will naturally be more densely crowded on the solid particles. It was decided to follow the procedure adopted in soil bacteriology (**15**), and to estimate the number per gram of *dry* mud.

(ii.) Some bacteria, such as the nitrogen-fixing bacteria and the nitrifying bacteria, do not grow readily on ordinary culture media, and the figures given below therefore do not include these.

(iii.) Slow-growing forms, such as certain Spirilla, form very small " pin-point " colonies, which are not easily seen with the naked eye when counts are made. For these reasons the bacterial content is probably underestimated.

The numerical work was supplemented by some descriptive and diagnostic study of the species isolated ; for this the ordinary bacteriological routine technique was adopted. In the course of this work many hundreds of different strains were isolated. Only a few of these could be identified as known species, and of the many unknown forms, only a few were studied in any detail.

TABLE I.

Estimations of Bacterial Content of Mud Samples, Station 10, Loch Striven Head.

	Depth of sub-sample in cm.	Agar Cultures, c.c. 0·5	0·5	0·1	0·1	Gelatine Cultures, c.c. 0·5	0·5	0·1	0·1	Average per c.c. of 1 : 100 dilution of wet sample.	Wt. of wet sample in gm.	No. of bacteria in thousands per gm. of wet mud.	Wt. of dry sample in gm.	No. of bacteria in thousands per gm. of dry mud.
16/4/30	0–1	255	330	86	40	207	330	187	88	635	1·10	58	0·37	171
	10–11	166	96	36	38	270	244	11	10	362	0·84	43	0·43	84
27/5/30	0–1	869	689	238	452	1005	940	379	241	2005	2·47	81	0·88	227
	2–3	673	463	190	241	611	443	214	93	1219	2·97	41	1·50	81
	6–7	139	247	153	88	221	225	114	91	532	1·72	31	0·92	57
	10–11	266	274	144	149	433	417	67	60	754	1·55	49	0·82	91

TABLE II.

Station 11.

16/4/30	0–1	158	186	39	38	285	226	29	46	419	0·86	49	0·14	300
	2–3	244	298	192	176	607	641	220	57	1014	1·88	54	0·58	175
	10–11	205	148	—	53	263	323	31	26	456	1·00	46	0·38	120
	25–26	107	162	37	29	195	146	51	45	321	1·06	30	0·44	73
27/5/30	0–1	576	684	226	139	431	244	148	95	1059	2·70	39	0·75	140
	2–3	596	517	241	203	*	507	295	126	1307	2·13	61	0·73	180
	10–11	324	237	84	118	326	518	74	38	716	1·28	56	0·39	184
	24–25	153	250	188	214	374	360	83	116	724	1·92	38	0·84	87

* Colonies too crowded for counting.

TABLE III.

Estimations of Bacterial Content of Mud Samples.

Station 7B.

	Depth of sub-sample in cm.	Agar Cultures, c.c. 1·0	1·0	0·5	0·1	Gelatine Cultures, c.c. 1·0	1·0	·05	0·1	Average per c.c. of 1 : 100 dilution of wet sample.	Wt. of wet sample in gm.	No. of bacteria in thousands per gm. of wet mud.	Wt. of dry sample in gm.	No. of bacteria in thousands per gm. of dry mud.
14/4/30	0–1	582	448	306	0	743	585	370	48	593	2·39	24	0·73	81
	10–11	434	547	230	56	353	432	195	18	435	0·97	45	0·39	110
	27–28	215	187	128	9	355	312	239	12	280	1·16	25	0·51	55
	Depth of sub-sample in cm.	Agar Cultures, c.c. 0·5	0·5	0·1	0·1	Gelatine Cultures, c.c. 0·5	0·5	0·1	0·1					
3/6/30	0–1	147	29	22	43	119	137	198	145	350	1·12	31	0·31	113
	1–2	72	62	34	29	133	37	—	108	206	0·94	22	0·30	69
	2–3	59	25	21	25	122	134	102	155	267	1·00	27	0·33	81
	3–4	40	27	31	38	81	—	77	110	212	0·69	31	0·23	92
	4–5	87	71	49	70	53	57	71	58	215	0·91	24	0·31	69
	6–7	33	17	18	60	96	129	81	53	202	0·71	29	0·24	84
	12–13	24	44	33	45	*	*	114	101	257	0·77	33	0·29	89
	20–21	27	47	34	50	124	119	84	47	221	0·80	28	0·32	69
	29–30	41	44	39	31	*	185	46	115	263	1·05	25	0·45	58

TABLE IV. Station 1B.

	Depth of sub-sample in cm.	Agar Cultures, c.c. 1·0	1·0	0·5	0·1	Gelatine Cultures, c.c. 1·0	1·0	0·5	0·1					
10/4/30	0–1	*	*	328	103	*	*	622	150	1002	2·24	44	0·93	107
	5–6	297	372	102	59	*	*	*	*	320	1·89	17	1·16	28
	10–11	*	*	120	35	*	*	168	68	325	1·11	29	0·70	46
	Depth of sub-sample in cm.	Agar Cultures, c.c. 0·5	0·5	0·1	0·1	Gelatine Cultures, c.c. 0·5	0·5	0·1	0·1					
27/5/30	0–1	334	343	105	111	220	189	131	109	642	1·97	33	0·91	70
	2–3	362	418	154	219	*	*	31	87	908	2·59	35	1·42	63
	6–7	301	—	151	205	539	—	181	181	1112	2·59	43	1·65	69
	10–11	418	—	93	70	466	—	116	239	1001	3·15	32	2·11	47

* Colonies too crowded for counting.

RESULTS.

(*a*) Quantitative Work.

Station 10, *Loch-head Type.* In the sample taken on April 16th, the number of bacteria at the surface of the mud was greater than at a depth

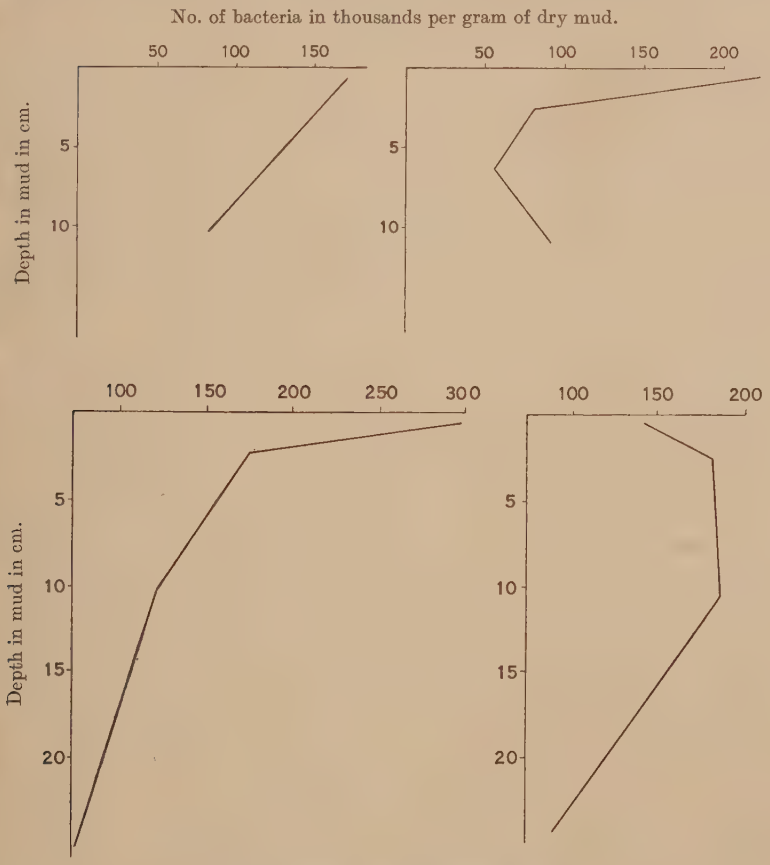

Fig. 1.—Bacterial Content of Marine Muds.
Above, Station 10, 16/4/30 and 27/5/30.
Below, Station 11, 16/4/30 and 27/5/30.

of 10–11 cm. (Fig. 1). A second sample, taken on May 27th, showed a surface increase from approximately 170,000 to 227,000 bacteria per gram of dry mud. It showed also a progressive decrease from the surface down to 6–7 cm., and an increase at 10–11 cm.; at this depth there was

relatively little change in the bacterial content in the interval between the first and second sampling (Table I).

Station 11, *Mid-loch Type.* The first sample taken on April 16th showed a progressive downward decrease in the number of bacteria. After five weeks, a second sample showed a surface decrease from 300,000 to 140,000, so that there were then fewer bacteria at the surface than at 3–4 cm. and at 10–11 cm. At 24–25 cm. there was a slight increase in numbers (Fig. 1 ; Table II).

Station 7*B*, *Deep-water Type.* At this station mud samples from the greatest depth of water were examined. The first sample, taken on April 14th, was marked by higher bacterial content at 10–11 cm. than at the surface : at 27 cm. the numbers were much lower. In order to determine more precisely the vertical variation in bacterial content, the second sample taken on June 3rd was divided into sub-samples at closer intervals. It was found that the number was highest at the surface, but there were two zones, at 3–4 cm. and 12–13 cm. respectively, where the bacterial content increased. Below that level the numbers gradually diminished (Fig. 2, Table III).

Station 1*B*, *Sandy Type.* The first sample, taken on April 10th, had a moderately high surface number, but at 5–6 cm. an abnormally low bacterial content—the lowest for any mud examined from this area ; there were almost twice as many bacteria per gram at 10–11 cm. as at 5–6 cm. The second sample, taken some six weeks later, showed a great decrease in the number of bacteria at the surface, a slight decrease at 2–3 cm., a slight increase at 6–7 cm., and a decrease at 10–11 cm. (Fig. 2, Table IV).

Summing up these results (see Table V), it is seen in general that the number of bacteria is in most cases greatest at the surface, that there is usually a progressive decrease with increase in depth, that the number fluctuates very widely near the surface of the mud, but that it remains fairly constant at the greatest depths for any given station.

(*b*) Description of Mud Bacteria.

In view of the large number of strains isolated, it was not found possible to study the physiology and morphology of all the forms. Certain species with special cultural or physiological characteristics were selected as types for further study, and those organisms having many characters in common were grouped together.

The marine muds examined were found to contain micro-organisms belonging to the following groups :—

(i.) The most frequent organisms were small bacilli similar to those found commonly in sea-water, and resembling the genera *Achromobacter*

Bergey and *Chromobacterium* Bergey (**1**), which appear to be common in fresh water. These organisms are true water bacteria.

TABLE V.

SUMMARY OF BACTERIAL CONTENT OF MUD. THE FIGURES REFER TO THE NUMBER OF BACTERIA IN THOUSANDS PER GRAM OF DRY MUD, APPROXIMATED TO THE NEAREST THOUSAND.

Depth in cm.	Station 10.		Station 11.		Station 7B.		Station 1B.	
	16/4/30	27/5/30	16/4/30	27/5/30	14/4/30	3/6/30	10/4/30	27/5/30
0–1	171	227	300	140	81	113	107	70
1–2						69		
2–3		81	175	180		81		63
3–4						92		
4–5						69		
5–6							28	
6–7		57				84		69
10–11	84	91	120	184	110		46	47
12–13						89		
20–21						69		
24–25				87				
25–26			73					
27–28					55			
29–30						58		

Morphologically, they are non-sporing and Gram-negative, and may be motile or non-motile. On agar they form moist, raised, more or less circular colonies similar to those of the colon-typhoid group, but they are usually slower-growing, and they vary in colour from white or cream to yellow or light brown. Physiologically, they were found to be extremely inert. They grow as readily on the ordinary peptone-containing media as on media prepared from fish extract, but they do not form indol from peptone, and do not readily ferment sugars, not even dextrose. They are thus physiologically much less active than terrestrial saprophytic bacteria under the experimental conditions tried ; it is of course possible that this group depends for its nutrition, not directly on the tissues of dead marine organisms, but on the simple nitrogenous compounds and carbohydrates dissolved in the water between the mud particles.

The bacteria of this class isolated from the mud samples have low oxygen requirements. The colonies in cultures used for the quantitative work were frequently found growing down into the medium away from the surface. Although they prefer anaerobic cultural conditions, they are facultative ærobes, for after repeated sub-culture they grow equally readily in well aerated parts of the culture medium. Most of these organisms were also able to utilise nitrates as a source of oxygen by reducing the nitrates to nitrites. Some strains carried the reduction a stage further, with evolution of gaseous nitrogen ; such denitrifiers were easily recognised because of the ease with which they reduced nitrates to nitrogen even in the

presence of some free oxygen. The occurrence of denitrification depends not only on the physiological specificity of the organism concerned, but

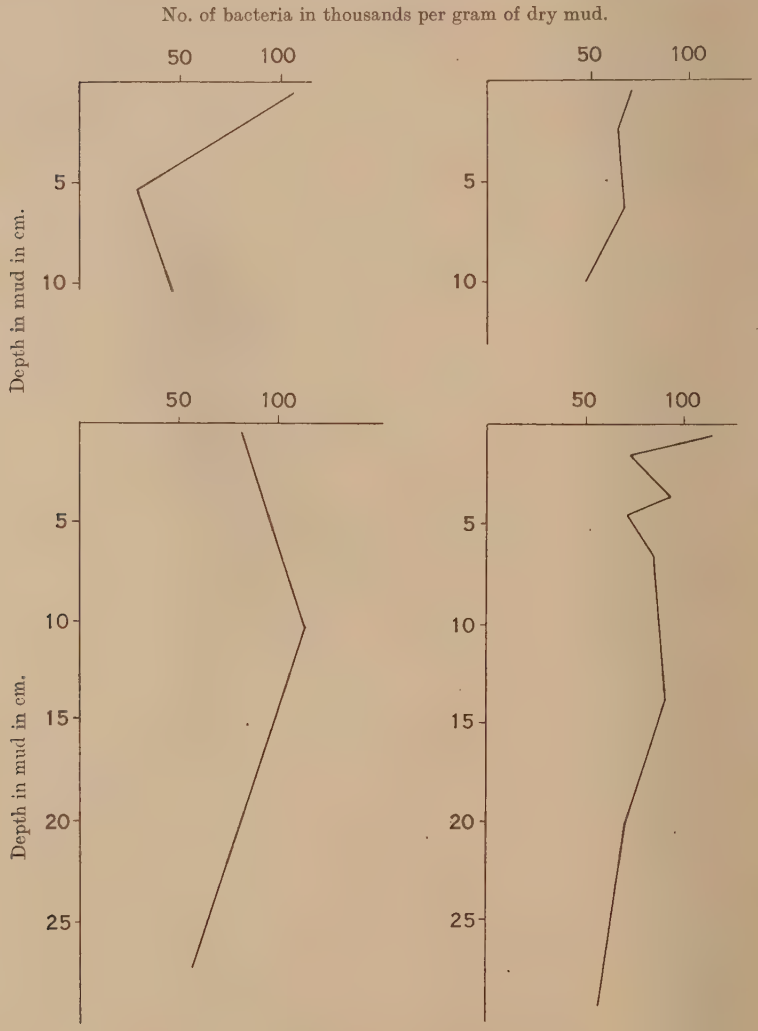

Fig. 2.—Bacterial Content of Marine Muds.
Above, Station 1B, 10/4/30 and 27/5/30.
Below, Station 7B, 14/4/30 and 3/6/30.

on such external conditions as temperature, hydrogen ion concentration and initial concentration of nitrate (8).

(ii.) There occurred in the samples a small proportion of small Gram-negative diplococci and sarcinæ forming cream, yellow or brown colonies. They resembled physiologically the first group, but were morphologically distinct. However, since morphologically bacteria are extremely plastic, it is possible that these are stages in the life history of members of the first group.

(iii.) The second group in order of numerical importance was found to consist of relatively large, spore-forming bacilli similar to the forms common in soil. Microscopically, they are often seen adhering in long chains. Culturally, they are recognised by their white slightly raised colonies, with a matt surface and irregular, dendritic or radiating outline. Physiologically they are more active than the preceding group, for they ferment the common sugars more readily; dextrose and sucrose are commonly attacked by these organisms, and a few are even lactose fermenters. Some strains produced indol from peptone. Under favourable conditions most of the members of this group reduced nitrates to nitrités, and a few reduced nitrates to gaseous nitrogen.

These organisms form a much larger proportion of the bacteria of the muds than of the water above, and they present many resemblances to the common spore-forming soil organisms. It is therefore necessary to consider whether they are species peculiar to marine muds, or whether they are terrigenous. It is not known whether they exist in the mud in the spore state or in the vegetative state.

(iv.) A small proportion of spirilla occurs. They grow only slowly on ordinary media, and the quantitative counts therefore often omit them. They may, however, be found in a mud dilution by first sedimenting the larger mud particles and then centrifuging the suspended micro-organisms and finer mud particles. Large colourless spirilla containing sulphur globules were occasionally found.

DISCUSSION OF RESULTS.

From the foregoing it is seen that in the Clyde Sea Area the bacterial content of the sea bottom is higher than that of sea-water, even of the water immediately overlying the mud. These results do not necessarily represent the total number of bacteria, since it is highly probable that there are many forms which do not grow on ordinary media, and therefore escape notice. The bacteria of the mud may be affected by the following factors :—

1. *Oxygen.* It appears that there is no free oxygen in the mud itself, and that oxygen cannot penetrate far below the surface. This has a marked effect upon the distribution of the fauna; Moore has shown elsewhere in this Journal (**10**) that the oxygen-loving Copepods are restricted to the

top few centimetres, while the Nematodes, on account of their lower oxygen requirements, can penetrate deeper. It is evident that bacteria are not as a class thus restricted, for they occur in very large numbers even at a depth of 30 cm. These bacteria thus live normally under anærobic conditions, though when cultures are made they grow quite readily in air. It is possible that the muds contain in addition many strict anærobes, whose presence would not be revealed by the ordinary counting technique. However, some preliminary experiments with anærobic cultures made from mud samples did not show any conspicuous anærobe.

The terms "ærobe" and "anærobe" have become increasingly unsatisfactory, since it has been shown that the difference is only one of degree, and that the so-called anærobic bacteria are really organisms which require a low oxygen pressure. Some of these so-called anærobes can utilise combined oxygen; the most important of these are the denitrifying bacteria, which will reduce nitrates, using the oxygen for respiration, and liberating the nitrogen as waste metabolite. There is no direct experimental evidence to show that denitrification occurs in muds.

Hitherto it has been thought that in temperate latitudes the destructive effect of denitrifying bacteria on the nitrate in the sea was negligible, for the following reasons:—(i.) the number of bacteria in the sea-water is relatively low (**7**), and of these only a certain number are denitrifiers, (ii.) free oxygen is present in sufficient amount to inhibit nitrate-destruction (**3**), and (iii.) the low temperature prevents denitrification (Gran, **3**). The last condition is the only one that applies to the muds, however, for the number of bacteria is extremely high, and the aeration insufficient. The hydrogen ion concentration, the low nitrate concentration, in fact, all conditions except temperature, favour denitrification, so that on theoretical grounds it would appear that loss of nitrate by bacterial action is much more likely to occur here than in the sea-water above.

2. *Hydrogen ion concentration.* There is a slight downward decrease in pH value in the muds (**10**), and there is also a downward decrease in the bacterial content. However, although many micro-organisms are extremely sensitive to changes in hydrogen ion concentration, the range of variation here is too slight to account for the progressive downward decrease in the number of bacteria.

3. *Temperature.* The rate of multiplication of bacteria increases with temperature up to the optimum for any given species. No data for the temperature of the muds are available, but the low temperature of the bottom waters—from 7° to 14° C. in Loch Striven—(**12**) suggests that the rate of multiplication of bacteria on the sea bottom is limited by this factor.

4. *Light Intensity.* With increasing depth of overlying water there

is diminished light intensity at the mud surface. This does not appear to have any effect on the bacterial numbers ; there is no apparent relation between the bacterial content at the mud surface and the depth of overlying water. This is confirmed by the fact that at the mud surface the numbers are usually greater. In any case, even the surface of the mud does not receive the short light waves, which are bactericidal in their action.

4. *Food Supply.* The most important factor affecting bacterial numbers is the amount of available foodstuffs. The chief source of food supply to the mud bacteria is the accumulated dead plankton. This is subject to seasonal variations, for the amount of organic matter deposited on the sea floor depends upon the flora and fauna in the waters above. In the lochs of the Clyde Sea Area the sedimentation of phytoplankton after the spring diatom increase is most important : Moore has shown (**10**) that this seasonal deposition of diatoms forms well-marked layers in the muds of undisturbed stations.

Such zones would be layers of intense bacterial activity, and would have a much higher bacterial content than the intervening layers ; these zones would continue to have a high bacterial content until such time as the food supply was exhausted by the bacteria or other organisms competing for the same food supply. That bacterial zonation occurs in the mud is shown diagrammatically in Figure 2, where at Station 7B there are between the surface and 7 cm. four zones of alternating high and low bacterial content. A more detailed examination of the mud samples would probably show a better correlation between the diatom layers and the zones of high bacterial content, but this so far has not been done ; it would be necessary to take many more samples, in order to ensure that such variations were not chance variations.

Although the surface numbers are high, they are irregular, and do not show any definite relation to the spring increase. For instance, the Lochhead and Mid-loch types show a great difference in bacterial numbers, though at these two places the spring increase is parallel, and of almost equal intensity.

In the deeper mud layers, the food supply is gradually exhausted, but the fauna becomes progressively reduced in numbers downwards, so that bacteria are in all probability the predominant organisms at the greatest depth.

It has already been pointed out that there are great fluctuations in bacterial numbers at the mud surface. These fluctuations would depend partly upon the varying amount of foodstuffs present, and also upon the numbers of organisms such as protozoa which feed upon the bacteria.

The spring diatom increase at all stations was over by April 1st, 1930 ; the accumulation of diatoms at the bottom then follows, the time interval

depending upon the depth of the water. An accumulation of plankton rain does not necessarily imply a sudden increase in the bacterial population at the bottom. Different species may develop according to the ordinary population curve with an initial lag period, a maximal growth period, and a period of decay due to diminished food supply and accumulation of toxic metabolic products. Different groups of bacteria may follow one another, but there is at present no experimental evidence for this. At the bottom of the sea, however, conditions are complex and cannot accurately be reproduced in the laboratory, so that it is difficult to gain exact knowledge of the growth and physiology of mud bacteria under natural conditions.

In conclusion, the writer wishes to acknowledge the help received from members of the Station staff at Millport.

SUMMARY.

1. The bacterial content of mud deposits in the Clyde Sea Area has been investigated.

2. The number of bacteria is found to decrease from the surface downwards.

3. The numbers fluctuate very much in the top mud layers, and there is some evidence of bacterial zonation.

4. In the deeper mud layers the bacterial content for any given station is fairly constant.

5. The predominant organisms were found to be water bacteria of the *Achromobacter* and *Chromobacterium* type, and large spore-forming bacilli similar to common soil bacteria.

6. The factors affecting the bacterial content of the muds are discussed.

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The Vertical Distribution of Marine Macroplankton. XI. Further Observations on Diurnal Changes.

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With 7 Figures in the Text.

On June 3rd–4th, 1926, a further series of observations was made on the vertical migrations of plankton animals by means of collections with the 2-metre stramin ring-trawl. Thus, with the observations made on one night in July, 1924 (**1**), and on two nights in June, 1925 (**2**), we now have data for four nights.

Since so few observations of this nature have been made at sea it seems desirable that the results obtained on this fourth night should be published.

The collections were made in the same way as on the previous collections and the log, giving full details of conditions at the time of collecting, has been given in a previous publication (**3**) in which the behaviour of the young fishes is described.

The collections are noteworthy for the very large numbers of animals caught, catches as large as over 120,000 organisms for a ten-minute haul being recorded. Swarms of *Calanus finmarchicus* and Crab zoeas were chiefly responsible for these high numbers. It is also interesting to record the presence in the catches at night of numbers of small Portunid crabs up to about a size of 4 mm. in carapace breadth. Of thirty-eight specimens examined all were *Portunus depurator*.* Small *Pandalina brevirostris* and Crangonids also migrated at night into layers well above the bottom. Many of the animals living in the upper water layers in the daytime were much nearer the surface on the morning of June 4th than they were on the previous afternoon. This is possibly due to the light intensity being cut down by the enormous swarms of Calanus and Crab zoeas present on June 4th ; it has already been shown that many of the young fishes were affected in the same way (**3**, p. 833).

On the whole, however, the results are so confirmatory of the other observations that it is unnecessary to deal with the data as fully as in the two previous papers. They are therefore here given only in the form of

* I am greatly indebted to Mr. R. Palmer for the identification of these young Portunids.

diagrams for the many species (Figs. 1 to 7, pp. 776 and 782) and as a Table setting out the complete catches of all organisms (Table I, pp. 783 and 784).

Comparison of Results of Four Series of Collections.

It is now possible to compare the results of the four series of collections and on pages 771 to 775 are given in summary form the types of behaviour shown by the more important species on the four nights in question, namely July 15–16th, 1924, June 17–18th, and 18–19th, 1925, and June 3–4th, 1926.

It must be realised that for most species we are dealing with a mixed population, composed of individuals of different ages and perhaps sexes; this point has been emphasised in a recent publication (**4**) in which the behaviour of Sagitta of different sizes is studied. It must also be borne in mind that, in the method of collecting, the catches from the different depths have not been made simultaneously (see **2**, pp. 81 and 82). We cannot hope, therefore, to attempt to draw conclusions on the fundamental factors controlling the behaviour of the animals. The time has now come when a number of simultaneous collections must be made in rapid succession during the hours of changing light intensity at dusk and dawn, and attention must be given to the behaviour of each stage of development of the species caught.

Nevertheless these four series of collections have given us a very good picture of how the larger plankton animals behave as a whole throughout the twenty-four hours in June and July in the waters off Plymouth.

After a careful study of the available data it is evident that whatever be the physical and chemical conditions of the environment that control the behaviour of the animals and provide the necessary stimuli, there are two factors inherent in the animals themselves which are largely responsible for the type of distribution shown by any species during the night, namely :—

1. The depth at which the animal has been living during the previous daylight.
2. The speed at which the animal is capable of swimming upwards.

In the report on the first night's observations in 1924 (**1**, p. 779) the types of behaviour shown by the different species were grouped under the following four headings :—

1. Those that definitely migrated to the surface at night from the deeper layers in which they dwelt by day, showing a very large increase in numbers on the surface with a corresponding decrease in the deeper layers.

2. Those that did not show a definite migration to the surface at night, but merely extended their distribution into the surface layers, which they avoided by day. In this case a diminution in numbers was shown at the region of maximum intensity in the daytime, so that the distribution from surface to deeper layers was more or less uniform.
3. Those forms whose daytime distribution altered little or not at all at night.
4. Those that showed a movement upwards from the bottom, appearing in large numbers at night at a level about 10 fathoms from the bottom.

Actually it seems probable now that these groups cannot be separated, but that one merges into the other and a complete gradation can be obtained in the different types of behaviour, depending largely on the depths at which the animals are living in the daylight and the rates at which they can swim upward. In the present paper the figures giving the diagrams of vertical distribution for the different species (Figs. 1–7, pp. 776 and 782) have been arranged in order to give more or less a complete gradation from a species such as Leuckartiara (=Turris) which migrates rapidly to the surface at dusk, to Mysids and other bottom-living animals which have time only to reach the layers up to 20 metres below the surface. Good examples also are given showing how in one group of animals differences in activity will produce different behaviour at night. The diagram in Figure 6, for instance, shows that Mysids, chiefly *Leptomysis gracilis*, do not apparently have time during the night to migrate in numbers above 20 metres, whereas *Anchialus agilis*, as its name implies, is able rapidly to migrate right to the surface in considerable numbers (Fig. 5). A comparison of these figures with Figures 5 and 6 in the previous publication (**1**) shows that in 1924 these two species of Mysids showed exactly the same difference in their behaviour, the diagrams for the same species being almost identical. Figure 7, p. 782, shows the behaviour of two Cumacean species* at night ; *Diastylis rostrata*, like Leptomysis, did not appear in numbers above 20 metres, but *Bodotria scorpioides*. like Anchialus, migrated right to the surface. It is interesting to record as on a previous occasion (**1**, p. 797) that many of the Diastylis taken above the bottom at night were females. The young swimming crabs also were able to mount right to the surface in the dark, whereas the young of *Pandalina brevirostris*, like Leptomysis, could not migrate in numbers above

* I am greatly indebted to Dr. W. T. Calman, F.R.S., and Dr. Isabella Gordon for the identification of these two species as *D. laevis* Norman (=*D. rostrata* (Goodsir) in "Plymouth Marine Fauna") and *Bodotria* (=*Cuma*) *scorpioides* (Montagu) (=*C. edwardsi* of Sars', "Crustacea of Norway"). *B. scorpioides* is recorded for the first time at Plymouth.

the 20-metre level. Many other examples can also be seen, such as *Themisto gracilipes* as opposed to bottom amphipods. It is possible even that some species, e.g. perhaps Bougainvillea and Steenstrupia, which show no marked change in their distribution during the night hours, are prevented from doing so by their inability to swim quickly.

A study of pages 771–775, in which the results for the three years are compared, shows that for a great number of species almost identical types of behaviour were shown for all three years. For other species differences were shown, the animals behaving differently in one year from the other two. In no case did a species behave in a different manner in all three years. It is noteworthy that the majority of species showing the same type of behaviour in all three years were those which lived deep down or very near the bottom in the daytime, and whose depth is limited by that of the bottom. For animals of this class power of movement may be said to be almost more of a conditioning factor in their type of distribution at night than such factors as light intensity. For animals living nearer the surface, however, daylight may control the ultimate night distribution more, in that it affects the daytime distribution of the animals, which are not so limited in the depths to which they descend as are those which normally live very near the bottom. It is therefore more likely that differences in behaviour will be shown by those animals living nearer the surface in the daytime (see **4**, p. 404).

Differences in vertical distribution due to season also may affect the behaviour of the animals ; Tomopteris, for instance, in June lives very near the bottom in the daytime and its diurnal behaviour consequently appears very different from that shown in July when they are well up in the water during daylight.

At the same time the different animals are not all quite consistent in their behaviour. For instance, it has been shown that Sagitta in 1925 (**4**, p. 404) was living very near the bottom in the daytime, possibly being forced down by strong light owing to the clearness of the water, whereas in 1924 and 1926 Sagitta was well up in the water in the daytime. There is no evidence, however, that some of the other animals were affected to so marked a degree. Such phenomena can only be understood when we have far more observations carried out in greater detail.

A comparison of the four nights shows also that more of the deep-living animals reached the layers up to the surface in the nights in June, 1925, than they did on either of the nights in July, 1924, or June, 1926. It seems possible that owing to the scarcity of plankton animals on the nights in 1925 their passage upward was less impeded by obstructing animals. The presence of other animals in large numbers must in itself cause modification of the behaviour to be expected of an animal in perfectly uninhabited water. Both avoiding reactions and feeding reactions must

tend to divert an animal from its pure responses to physical and chemical environmental stimuli.

Summary of Observations on Four Nights.

*Steenstrupia nutans.** 1924. "No marked vertical movements at night": numbers rather low.

1925. First night—numbers rather low.
Second night: "in the dark there had been a decided movement into the layers above 20 metres": did not extend in numbers much above 12 m.

1926. No marked vertical movement at night; slightly higher in water at dawn.

Leuckartiara octona.† 1924. "By 9 p.m. the majority were caught above a depth of 10 metres, and at midnight they were taken in greatest numbers right at the surface."

1925. "On both nights—but more markedly on the first—there seems to have been an active migration to the surface itself at dusk, followed by a downward movement at night in the dark and a further upward migration at dawn."

1926. Although the numbers rather point to encountering horizontal swarms there was a marked migration to surface at dusk, followed by a descent in the dark and a further upward migration at dawn. The medusæ remained high in the water the following day.

Obelia sp. 1924. "It cannot be said that there was any marked movement towards the surface at night."

1925. "The catches of Obelia were very small, and appear to indicate little co-ordinated movement."

1926. Entirely absent at night: no indication of movement at dusk.

Phialidium sp. 1924. "showed no signs of being affected by changes in light intensity."

1925. "There appear to be definite indications of an upward movement on the part of these medusæ at night."

1926. No very marked movement at night shown.

Cosmetira pilosella. 1924. "it would seem that here is shown a definite migration to the surface at night." Numbers rather low: present at surface at dusk.

1925. On both nights were evenly distributed from surface downwards in the dark: no movement at dusk.

1926. Same as 1925.

* All specific names used are those adopted in the *Plymouth Marine Fauna*, 2nd edition. 1931. Previously recorded as *S. rubra*. † = *Turris pileata* in previous papers.

Saphenia gracilis. 1924. "Present at the surface both at 9 p.m. and at midnight, and absent from there at other times." Numbers low.

1925. "the impression gained is that in its diurnal behaviour Saphenia resembled Cosmetira, except that in the daytime it went possibly deeper."

1926. Resembled Cosmetira in its behaviour, except fewer at surface itself.

Tomopteris helgolandica. 1924. Present in numbers right at the surface at dusk and at night, but they were already high in the water in the daytime, when the region of maximum abundance was at about 20 metres.

1925. On both nights Tomopteris appeared in numbers on the upper layers at dark and dawn, being probably very near the bottom in the daylight.

1926. Very similar behaviour to 1925, the majority probably living very near the bottom in the daytime.

Sagitta sp. These have been dealt with separately in another publication (**4**), the populations having been divided up into different size groups. There was, however, a very definite difference in the behaviour in 1925 from that shown in 1924 and in 1926. This would seem to be explained by the fact that in the daytime in 1925 the Sagitta were almost on the bottom, whereas in the other two years they were well up in the water layers in daylight.

Calanus finmarchicus. 1924. A definite migration to the surface at dusk, followed by an even distribution from the surface downwards in the dark.

1925. "By dusk there had been a marked upward movement in the water. . . . In the dark they were mostly distributed between the surface and 25 metres. The movements . . . were not as marked on the second night as on the first."

1926. Adult females: Slight upward movement at dusk followed by fairly even distribution from surface downwards in the dark. Larger concentration at 5 metres at dawn.

Adult males: Being lower in the water in daytime than the females hardly any had reached the surface by dusk, and the majority were still deep in the water at night.

Candacia armata. 1924. Definite migration to the surface at dusk, followed by fairly even distribution at night.

1925. Definite migration to the surface in the dark, but not at dusk, as the majority were living very deep down in the daytime.

1926. Same as 1925.

Mysids : Mostly *Leptomysis gracilis.*

1924. Appeared in larger numbers at night, but majority did not rise above 20 metres.

1925. Similar behaviour to 1924 but rather more taken in surface layers.

1926. Behaviour same as in 1924.

Anchialus agilis. 1924. "Unlike the other Mysids, which never rose much above 20 metres from the bottom, this form exhibited a very sudden migration towards midnight right to the surface."

1925. Similar to 1924 though fewer at surface and more evenly distributed.

1926. Behaviour same as in 1924.

Cumaceans. Showed the same type of behaviour in all three years, appearing only in upper layers at night. It was noticeable that the Diastylis species, probably *D. rostrata*, were not caught in numbers above 20 m. at night, but that Bodotria sp. were able to reach the surface (see page 769).

*Themisto gracilipes.** 1924. "A marked migration to the surface at midnight."

1925. Very rare.

1926. Same behaviour as 1924.

Apherusa sp. 1924. "Exhibited no marked movement at night."

1925. Marked movement on both nights, being evenly distributed from surface downwards.

1926. Probably same behaviour as 1925, though numbers at night rather low.

Bottom Amphipods showed same type of behaviour in all three years, appearing in upper layers at night only.

Pandalid larvæ. 1924. "Did not . . . show any marked change. . . . There is perhaps a slight tendency to rise at midnight, but the larvæ never appeared in numbers in the surface layers."

1925. There was a definite indication of an extension into the upper layers at night.

1926. Rather similar behaviour as in 1924.

Crangonid larvæ and *post-larvæ.* 1924. Appeared in upper layers at night, but were still most abundant below 20 metres.

1925. Same as 1924. but larger numbers extending to surface.

1926. Same as 1925.

Phyllosoma larvæ. 1924. Marked movement right to surface by dusk : but entirely absent at night.

* *Parathemisto oblivia* in "Plymouth Marine Fauna."

1925. Same as 1924 : still present throughout upper water layers at night.

1926. Same as 1925.

Galatheid larvæ. 1924. " At dusk and at midnight they tended to become more evenly distributed from the upper layers downwards, but were at no time very abundant on the surface."

1925. Upper layers filled up right to the surface at night.

1926. Same as 1925 but not so marked, as they were living higher in the water in the daytime.

Galatheid post-larvæ. 1924. Appeared in numbers at night in upper layers, but still most abundant below 20 metres.

1925. In numbers right to surface itself at night.

1926. In numbers right to surface itself at night.

Porcellana zoeas. 1924. " had spread out by 9.30 p.m. into all layers, and were still distributed in this manner at midnight."

1925. " The midnight behaviour on these two nights was apparently very unco-ordinated, but may possibly have been upset by the presence of swarms."

1926. Same as 1924, but more massed in upper layers in dark.

Porcellana post-larvæ. 1926. Similar behaviour to that of Galatheid post-larvæ, but rather slower going down at dawn.

Upogebia larvæ. 1924. " By 9 p.m. they were present in all layers from the surface to 30 metres. At midnight by far the majority were taken on the surface."

1925. " By dusk they were extending their distribution up to between 10 and 15 m., and in the dark they were most abundant actually at the surface." On second night " the surface itself was not fully populated as on the previous night."

1926. By dusk many had already reached surface, and at night biggest catch was just below the surface at 4 metres.

Pagurid larvæ. 1924. " There was a very slight rise at midnight, but no increase in numbers at the surface."

1925. A very marked movement, the surface layers being filled from the surface downwards at night.

1926. Definite extension into surface layers at night.

Pagurid glaucothoë. 1924. " At midnight they were taken on the surface, but in greatest numbers at 20 and 30 metres."

1925. " In the dark they moved up into the upper water layers as far as the surface itself, though the largest catches were still below 25 m."

1926. Same behaviour as 1924.

Crab zoeas (mostly Portunids). 1924. "Extending into the upper layers and surface at dusk and midnight."

1925. "A considerable increase in numbers in the layers above 15 to 20 m. on both nights, an increase which was pronounced also at the surface itself."

1926. Same as 1924.

Crab megalopas (mostly Portunids). 1924. "At midnight . . . appeared on the surface in large numbers, being distributed from top to bottom."

1925. A marked movement to the surface in the dark on both nights.

1926. A marked movement to the surface in the dark. Although at the surface in the daylight they were mostly below 10 to 15 metres at dusk.

SUMMARY.

1. Details are given of the results of a series of hauls with the 2-metre stramin ring-trawl fished at six different depths in daylight, at dusk, in the dark, at dawn and again in daylight on June 3rd–4th, 1926.

2. A comparison is given of the results obtained on the four nights July 15–16th, 1924; June 17–18–19th, 1925, and June 3rd–4th, 1926.

3. The importance is stressed of the effects on the type of behaviour shown by any animal produced by the depth at which it is living in the daylight and the speed at which it can swim upwards.

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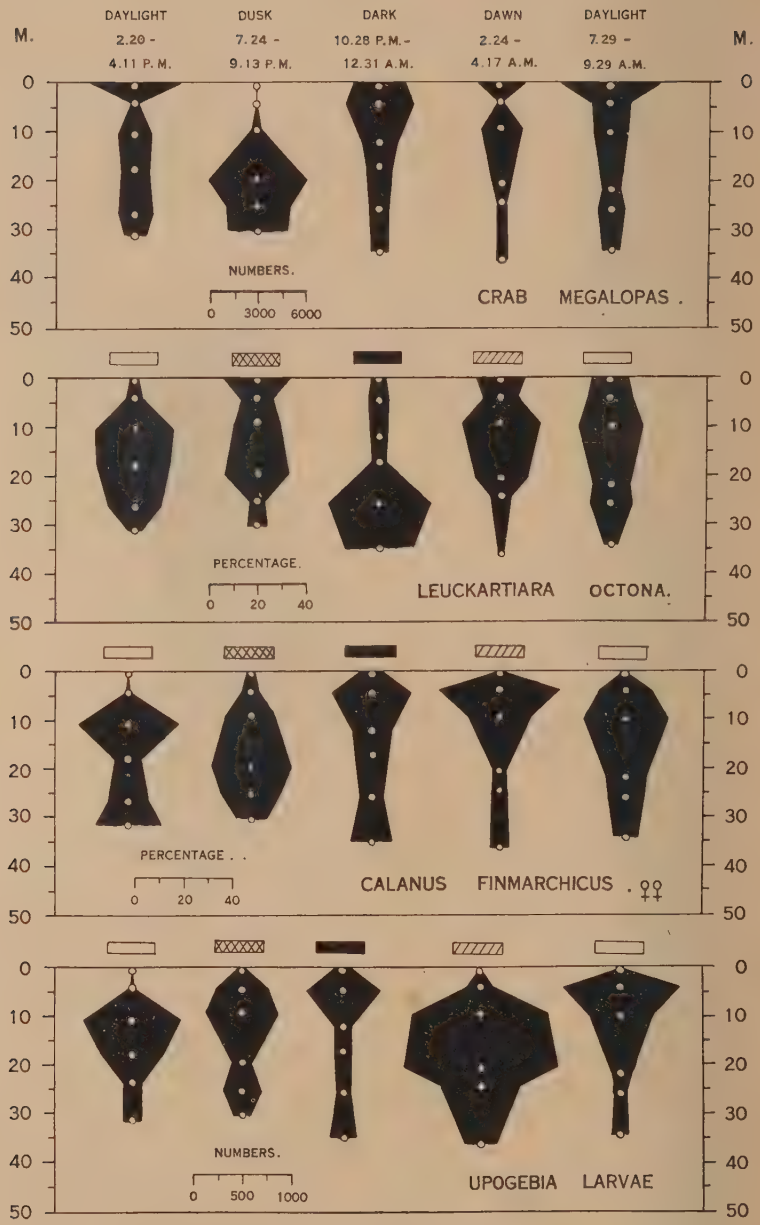

FIG. 1.—The vertical distribution of Crab megalopas (mostly Portunids), *Leuckartiara octona* (=Turris), *Calanus finmarchicus* adult females, and Upogebia larvæ at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

FIG. 2.—The vertical distribution of Porcellana zoeas, Crab zoeas (mostly Portunids), Galatheid larvæ, and Pandalid larvæ, at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," "dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

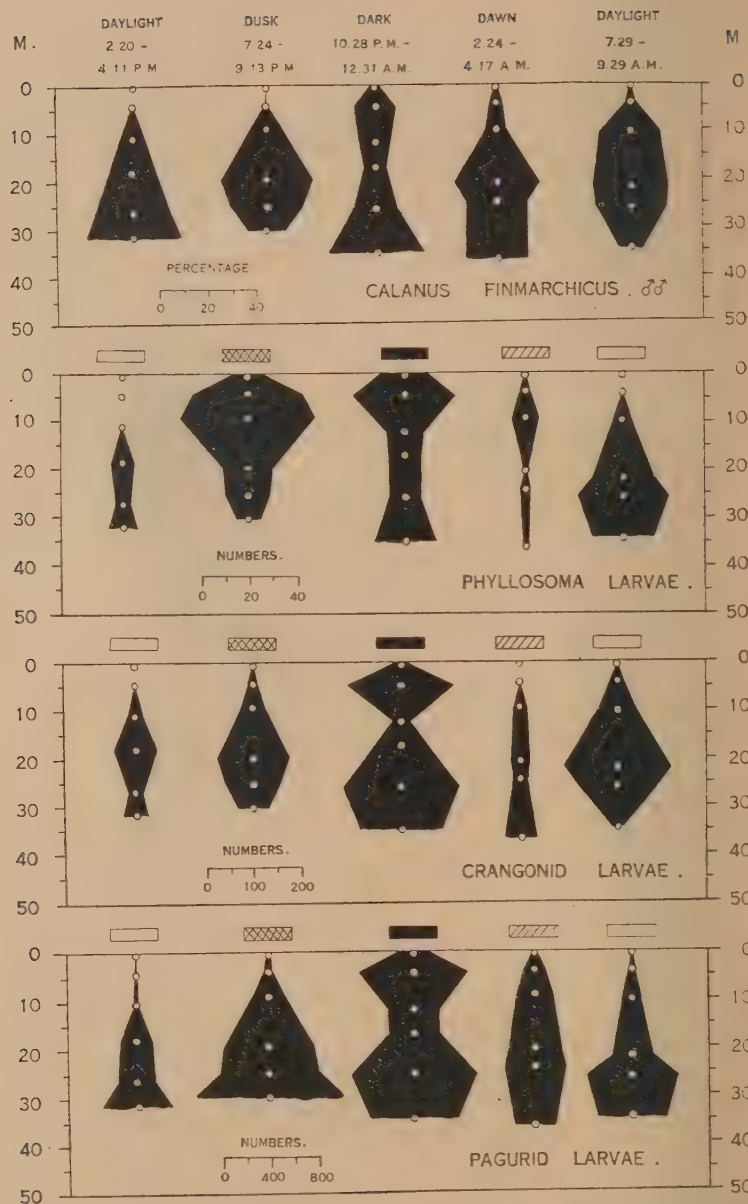

FIG. 3.—The vertical distribution of *Calanus finmarchicus* adult males, Phyllosoma larvæ, Crangonid larvæ, and Pagurid larvæ at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," "dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

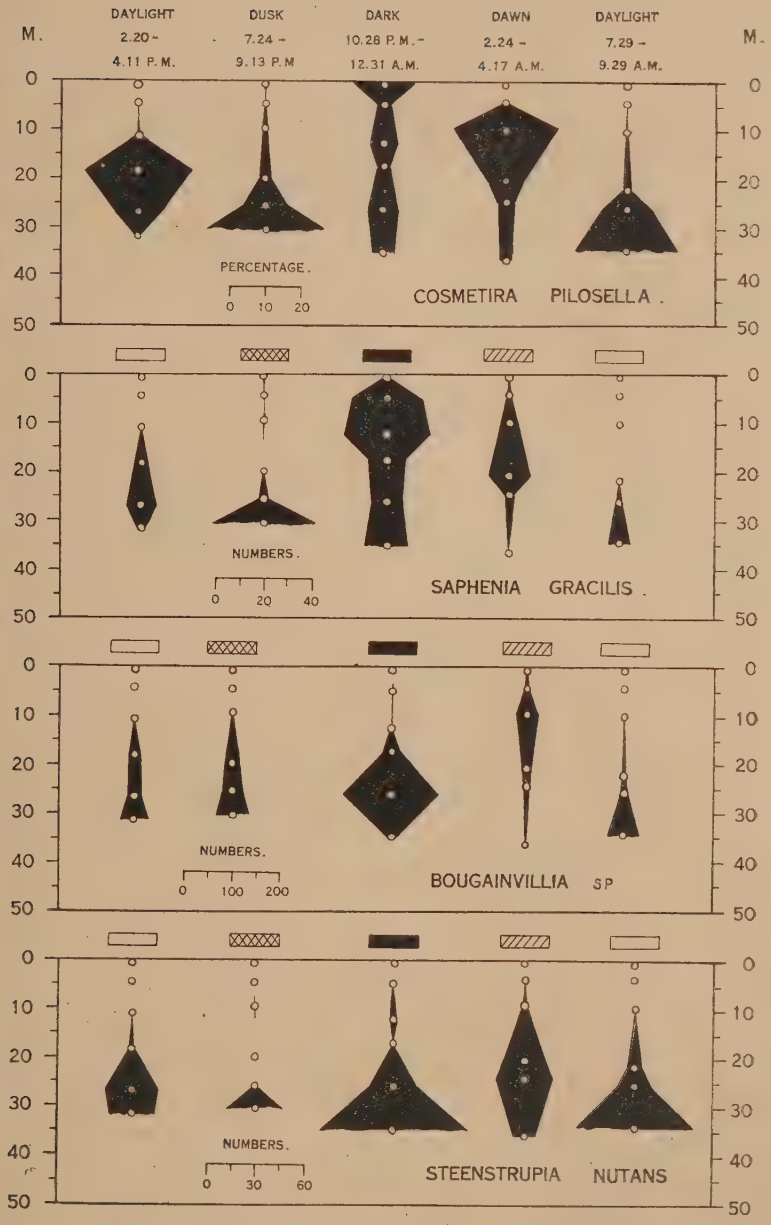

FIG. 4.—The vertical distribution of *Cosmetira pilosella, Saphenia gracilis*, Bougainvillea sp., and *Steenstrupia nutans* at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," "dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

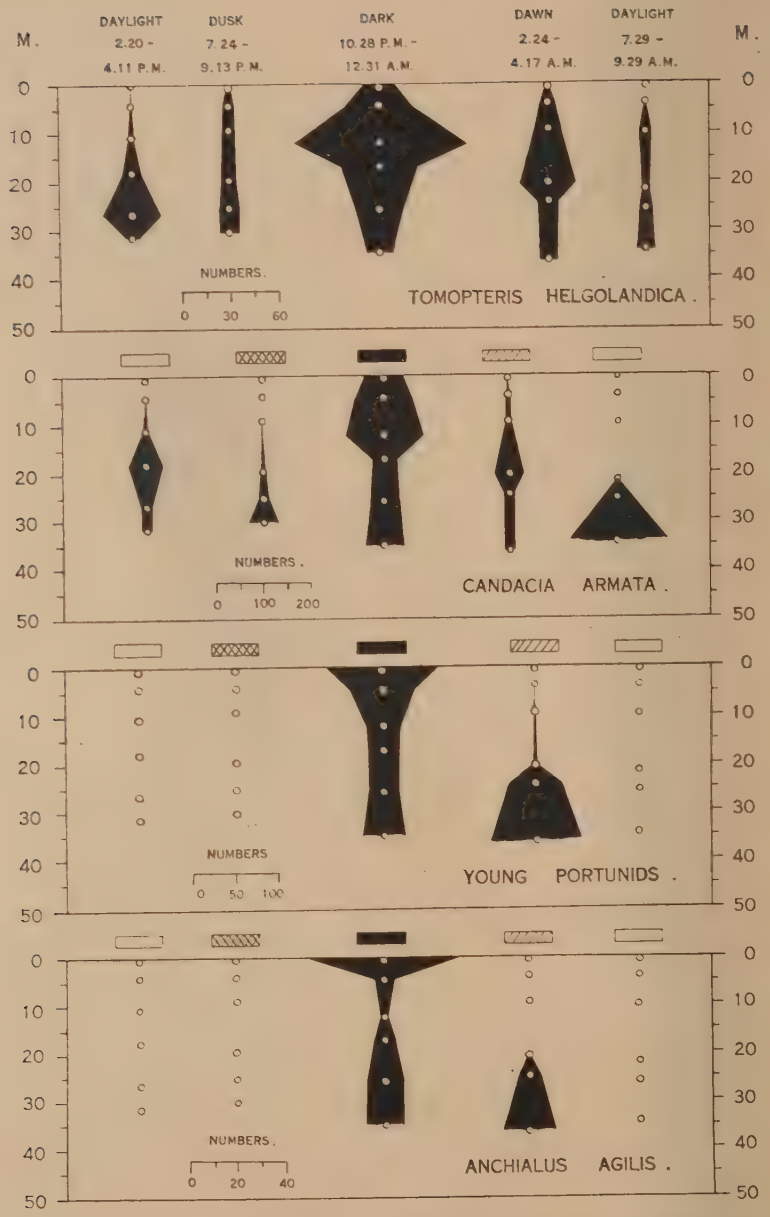

FIG. 5.—The vertical distribution of *Tomopteris helgolandica*, *Candacia armata*, Young Portunids, and *Anchialus agilis* at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," "dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

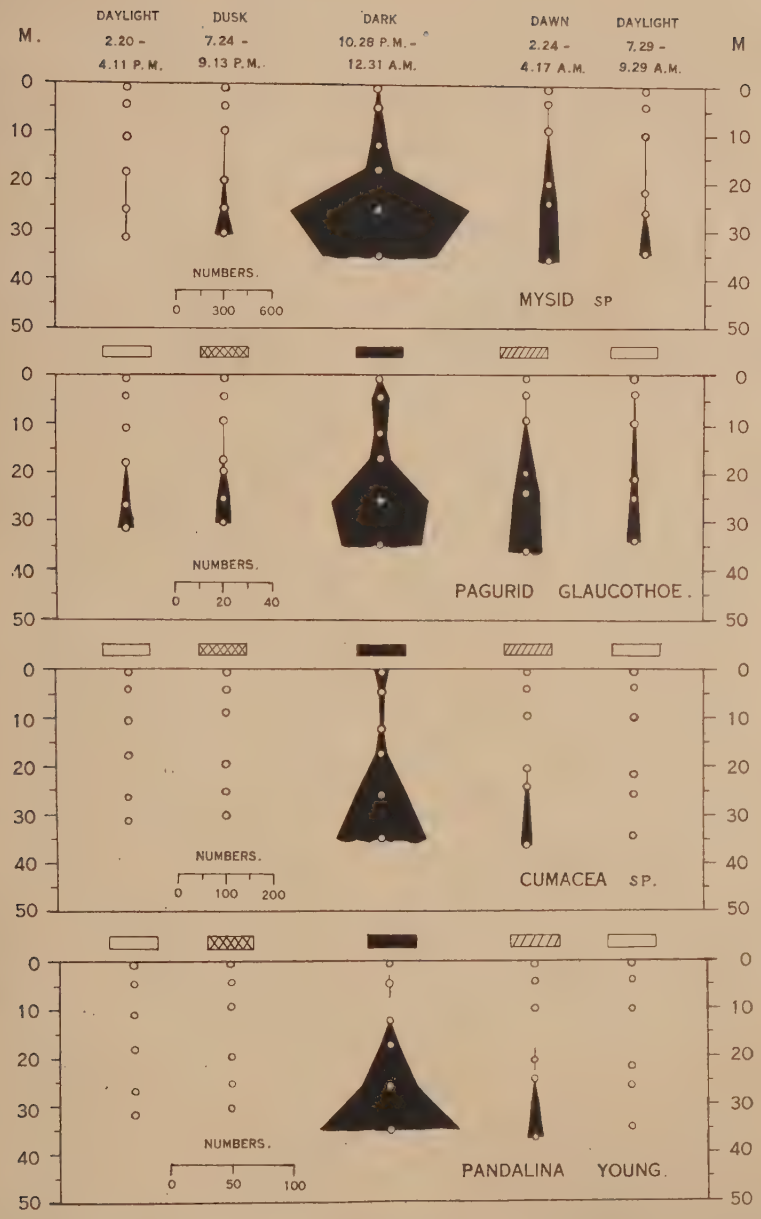

FIG. 6.—The vertical distribution of Mysids (chiefly *Leptomysis gracilis*), Pagurid glaucothoë, Cumacea, and young *Pandalina brevirostris* at the times shown on June 3rd–4th, 1926. The plain, cross-hatched, black, and shaded rectangles represent "daylight," "dusk," "dark," and "dawn" respectively. The white spots and black circles indicate the average depths at which hauls were taken.

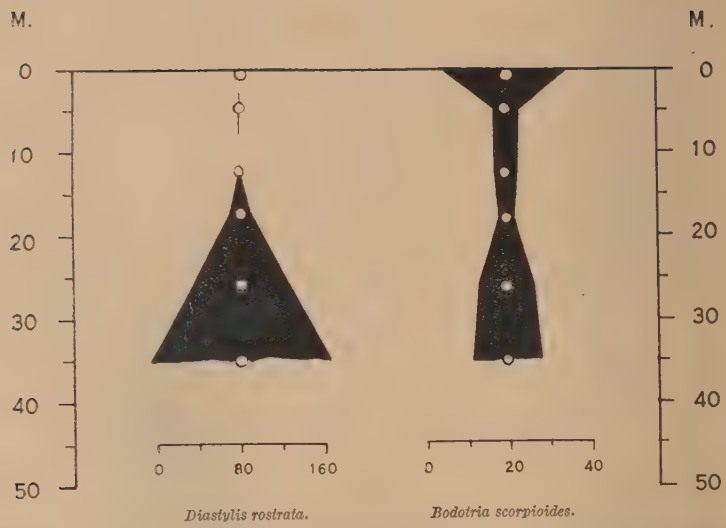

FIG. 7.—The vertical distribution of *Diastylis rostrata* (left) and *Bodotria scorpioides* (right) in the dark on June 3rd–4th, 1926. The white spots and black circles indicate the average depths at which hauls were taken. The scales give the actual numbers caught.

NOTES ON TABLE I.

SERIES 1.

Leuckartiara octona. At surface and 4·25 m. in first daylight series these were all small medusæ; the larger medusæ being in the deeper layers.
Tomopteris helgolandica from surface, 4·25 and 10·75 m., were all in a damaged state.
Nyctiphanes. Series one—all juvenile.
Mysids were very small stages.

SERIES 2.

Crab zoeas. Surface and 4·4 m. Mostly very small specimens.
Galatheid l. Surface, small.
Porcellana zoea. Surface, small.
Pandalid l. 4·4 m. Very small.
Crangonid l. 4·4 m. Very small.
Hyperiid at surface = *H. alba.*
Nyctiphanes. All juvenile.

SERIES 3.

Leuckartiara octona. Surface. Small.
4·6 m. Half small.
12·3 m. Most small.
11 m. Small.
rest=some small.

Young Portunids. Thirty-eight examined at 25·9 m. by R. Palmer= all *P. depurator.*

Young crangons up to 15 mm. long. At 25·9 there were two and at 35 m. three *Philocerus bispinosus* in berry 12 and 15 mm. long.

SERIES 5.

Leuckartiara octona. Surface to 10 m. Many small: larger deeper.

TABLE I. Ring-Trawl Catches. June 3rd–4th, 1926.

Time.	Depth in metres.	Steenstrupia nutans.	Slabberia halterata.	Bougainvillea sp.	Amphinema dinema.	Leuckartiara octona.	Cosmetira pilosella.	Obelia sp.	Phialidium sp.	Saphenia gracilis.	Cyanea lamarcki.	Pleurobrachia pileus.	Peachia larvae.	Arachnactis larvae.	Tomopteris helgolandica.	Poecilochætus larvae.	Sagitta elegans.	Sagitta setosa.	Calanus finmarchicus ♀	Calanus finmarchicus ♂	Calanus finmarchicus St. V.	Metridia lucens.	Candacia armata.	Anomalocera Patersoni.	Labidocera Wollastoni.	Caligus sp.	Mysid spp.	Anchialus agilis.	Haplostylus Normani.	Nyctiphanes.
4.1 p.m.	*S.*	–	–	–	–	7	–	–	90	–	–	–	3	–	1	–	40	13	117	6	–	–	–	2	2	–	–	–	–	–
3.44 „	*4·3*	–	–	–	–	12	–	–	320	–	–	–	3	–	1	–	92	49	2,000	43	107	–	–	–	20	1	–	–	–	–
3.25 „	*10·8*	–	–	2	3	58	35	50	790	–	–	–	4	1	4	–	1028	92	40,437	3,145	1,338	–	10	2	100	3	–	–	–	3
3.3 „	*18*	3	–	27	6	57	2271	4000	2360	6	–	–	7	1	12	4	1117	83	10,876	6,296	1,908	–	70	1	?	2	3	–	–	4
2.42 „	*26·6*	33	1	29	4	38	982	2700	3710	12	–	12	16	1	36	4	3784	167	16,585	10,120	1,405	–	20	–	50	1	5	–	–	3
2.20 „	*31·5*	28	2	60	2	10	119	810	1340	3	–	13	8	2	8	2	4090	120	26,441	12,591	2,938	–	20	–	140	2	4	–	–	8
9.3 p.m.	*S.*	–	–	–	–	393	33	–	120	1	3	1	–	–	2	–	513	677	3,046	90	1,344	–	–	–	10	8	–	–	–	–
8.46 „	*4·4*	–	–	–	–	183	5	–	190	–	2	–	–	–	6	–	392	288	5,474	805	1,771	–	–	1	–	7	–	–	–	–
8.28 „	*9·3*	1	–	3	–	271	38	–	310	1	1	–	3	–	7	1	755	745	16,832	7,088	5,610	–	–	3	20	3	1	–	–	–
8.9 „	*19·7*	–	–	31	–	373	140	20	320	1	–	1	7	1	9	3	1318	252	26,603	18,929	5,628	–	10	–	70	2	8	–	–	7
7.48 „	*25·3*	1	–	43	–	83	296	220	1520	4	1	6	21	1	13	–	766	464	20,401	14,572	1,457	–	20	–	10	4	29	–	–	1
7.24 „	*30·3*	35	1	63	3	118	1249	3730	4150	42	–	19	43	–	12	2	1530	550	10,597	7,308	365	–	60	2	10	2	101	–	–	3
12.21 a.m.	*S.*	–	–	–	–	11	848	–	30	1	1	2	–	–	18	–	143	287	13,798	2,123	1,769	–	80	2	–	1	2	63	4	9
12.1 „	*4·6*	–	1	4	–	17	199	–	380	29	–	9	3	1	41	1	188	452	52,805	10,268	10,267	–	120	3	–	2	30	8	2	72
11.36 p.m.	*12·3*	4	–	5		14	402	–	840	35	3	17	11	2	105	1	108	412	27,276	8,142	5,292	–	160	4	20	1	104	3	4	77
11.13 „	*17·2*	1	–	33	–	11	167	–	550	15	–	10	5	–	47	2	156	264	23,991	5,511	2,918	30	50	3	30	1	172	9	4	47
10.51 „	*25·9*	29	1	196	–	76	440	–	910	13	1	12	11	–	30	3	124	317	17,039	11,025	5,346	20	60	4	20	–	1106	16	1	83
10.28 „	*35*	91	1	15	–	52	292	–	1110	18	–	9	10	–	15	2	456	604	28,359	23,948	10,713	10	80	8	30	1	705	16	1	75
4.7 a.m.	*S.*	–	–	–	–	25	–	–	–	–	1	–	–	–	1	–	257	93	2,141	67	22	–	–	–	–	–	–	–	–	–
3.49 „	*4*	–	–	10	–	18	4	–	40	2	–	–	–	–	9	–	454	116	44,582	929	939	–	10	–	–	2	–	–	–	1
3.29 „	*9·5*	3	–	49	1	40	577	–	750	7	–	–	8	2	17	–	1152	338	23,842	1,340	1,608	–	10	–	20	1	4	–	–	5
3.8 „	*20·5*	27	1	18	2	27	196	1550	2310	17	–	2	17	–	35	3	993	207	5,630	5,755	1,125	–	60	–	100	2	68	–	–	17
2.47 „	*24·4*	35	1	11	–	12	92	460	720	3	–	3	6	1	13	1	388	172	6,458	4,066	1,436	–	20	–	10	2	107	9	1	16
2.24 „	*36·3*	13	2	8	1	3	61	390	170	–	–	3	2	–	9	–	507	72	7,118	4,163	2,149	20	20	–	60	1	129	21	–	8
9.19 a.m.	*S.*	–	–	–	–	123	–	–	30	–	–	–	–	–	–	–	117	13	529	76	25	–	–	60	20	–	–	–	–	1
8.59 „	*4*	–	–	–	–	156	–	–	10	–	–	1	–	–	–	–	347	244	37,375	3,602	4,053	–	–	3	50	1	–	–	–	2
8.38 „	*10*	–	–	–	–	223	9	–	100	–	1	4	–	–	7	–	852	107	58,660	19,003	4,957	–	–	–	20	2	–	–	–	7
8.17 „	*21·8*	9	–	10	–	120	61	20	120	–	5	4	1	–	4	1	1426	194	29,101	23,965	3,994	–	–	–	60	–	8	–	–	94
7.52 „	*25·7*	21	–	17	–	149	432	60	980	3	5	9	4	–	8	1	2428	272	25,327	22,347	1,986	–	60	–	–	1	11	–	–	48
7.29 „	*34·3*	72	1	61	–	43	864	220	1100	9	–	13	8	–	13	–	2112	808	17,563	8,376	1,081	–	200	1	–	2	63	–	–	33

TABLE I.—*Continued.*

Time.	*Depth in metres.*	Diastylis rostrata.	Bodotria scorpioides.	Themisto gracilipes.	Apherusa sp.	Bottom Amphipods.	Pandalid larvæ.	Pandalid post-larvæ.	Pandalina brevirostris.	Spirontocaris larvæ.	Leander larvæ.	Crangonid larvæ.	Pontophilus spinosus l.	Young Crangonids.	Palinurus phyllosoma.	Galatheid larvæ.	Galatheid post-larvæ.	Porcellana zoeas.	Porcellana post-larvæ.	Upogebia larvæ.	Pagurid larvæ.	Pagurid Glaucothöe.	Young Portunids.	Crab zoeas.	Crab megalopas.	Corystes megalopas.	Echinospira larvæ.	Limacina retroversa.	Total Organisms.
4.1 p.m.	*S.*	–	–	–	·	–	12	–		–	9	–	–	–	–	–	–	40	–	–	–	–	–	1,160	5490	–	–	–	**6,992**
3.44 ,,	*4·3*	–	–	–	–	–	280	–	–	–	9	–	–	–	–	130	–	180	–	50	10	–	–	4,200	530	–	–	–	**8,037**
3.25 ,,	*10·8*	–	–	·	10	–	670	–	·	140	25	30	2	–	–	2670	–	1020	10	1010	10	–	–	16,080	2030	–	10	–	**70,822**
3.3 ,,	*18*	–	–	1	40	–	830	–	–	60	5	90	26	–	10	5210	–	2360	10	680	260	–	–	8,980	1820	–	360	50	**49,906**
2.42 ,,	*26·6*	–	–	–	60	–	1180	2	–	70	10	30	16	–	6	5420	–	2200	30	200	310	3	–	7,870	2150	–	150	–	**59,425**
2.20 ,,	*31·5*	–	–	1	50	–	680	–	–	20	3	50	24	–	12	6210	–	2280	60	210	580	6	–	7,840	1490	–	20	–	**68,297**
9.3 p.m.	*S.*	·	–	1	–	–	·	–	–	–	3	–	–	–	12	180	–	1820	–	110	20	–	–	7,500	150	–	–	–	**16,037**
8.46 ,,	*4·4*	–	–	–	–	–	10		–		4	20	–	–	46	510	–	1750	–	560	70	–	·	3,910	90	–	10	–	**16,104**
8.28 ,,	*9·3*	–	–	–	150	–	270	–	–	–	19	50	–	–	56	3240	–	3130	20	750	260	–	–	12,720	650	–	40	10	**53,058**
8.9 ,,	*19·7*	–	–	–	170	–	1210	20	–	40	3	150	17	–	21	5580	10	2190	50	210	760	1	–	17,890	6230	–	70	20	**88,375**
7.48 ,,	*25·3*	–	–	2	220	–	880	–	–	40	8	110	4	–	19	5770	10	2200	90	400	820	5	–	10,790	4010	–	30	20	**65,361**
7.24 ,,	*30·3*	–	–	4	350	–	800	–	–	30	5	60	15	–	10	3920	10	2860	180	190	1220	6	–	7,250	3770	–	10	10	**50,695**
12.21 a.m.	*S.*	–	29	21	90	1	210	4	–	–	5	30	2	1	19	2220	160	1710	80	160	230	1	137	9,260	3370	1	20	40	**36,993**
12.1 ,,	*4·6*	2	6	4	40	7	780	18	1	60	11	220	6	34	42	6900	80	6180	240	760	900	7	82	25,700	4270	9	20	20	**121,301**
11.36 p.m.	*12·3*	–	5	–	60	5	1000	11	2	10	4	40	9	18	16	4060	220	3640	160	170	420	5	42	14,850	2210	14	50	20	**70,083**
11.13 ,,	*17·2*	21	3	3	–	3	620	12	15	10	5	110	9	42	14	3270	150	1540	90	170	420	9	40	10,340	1660	15	30	10	**95,680**
10.51 ,,	*25·9*	90	13	1	40	11	1470	15	47	40	–	240	30	44	13	5260	350	2230	220	150	1040	40	38	12,140	1240	12	50	10	**61,717**
10.28 ,,	*35*	170	16	2	–	21	1110	11	116	50	3	170	15	55	25	5070	320	1910	220	260	740	33	53	8,280	1140	14	90	–	**86,515**
4.7 a.m.	*S.*	–	–	–	20	–	20	–	–	–	10	–	–	–	–	50	–	1660	–	–	20	–	–	980	2800	–	–	–	**8,167**
3.49 ,,	*4*	–	–	–	50	–	40	–	–	–	9	–	–	–	4	1120	–	2960	10	270	170	–	–	5,260	440	–	–	–	**57,449**
3.29 ,,	*9·5*	–	–	–	70	–	310	–	–	30	11	30	5	–	11	2360	–	3020	120	1420	290	1	2	10,940	2540	–	70	–	**50,964**
3.8 ,,	*20·5*	–	1	–	30	2	430	1	1	30	4	40	4	–	1	3390	40	2870	480	1580	440	8	4	7,030	1180	–	90	–	**35,818**
2.47 ,,	*24·4*	2	1	–	40	16	380	2	–	30	4	30	5	19	4	4540	210	4140	470	790	490	11	65	6,510	680	3	30	–	**32,515**
2.24 ,,	*36·3*	12	5	2	20	29	390	3	13	–	1	70	4	17	1	2410	140	1240	360	330	330	14	112	3,720	850	5	30	–	**25,038**
9.19 a.m.	*S.*	–	–	–	30	–	10	–	–	–	5	–	–	–	–	380	–	1190	–	40	–	–	–	3,310	6080	–	–	–	**12,039**
8.59 ,,	*4*	–	–	–	90	–	1200	–	–	80	7	30	2	–	–	4630	–	7650	–	1190	60	–	–	17,570	2440	–	30	–	**80,823**
8.38 ,,	*10*	–	–	–	120	–	1820	–	–	40	2	80	15	–	8	8360	–	6820	–	660	160	1	–	16,920	2180	–	60	40	**121,238**
8·17 ,,	*21·8*	–	–	–	200	–	720	11	–	–	1	220	14	–	25	5300	20	3660	100	220	340	2	–	10,360	1160	–	20	–	**81,570**
7.52 ,,	*25·7*	–	–	2	280	–	720	16	–	–	2	160	9	–	38	3660	20	2880	60	140	760	3	–	5,120	1620	–	20	–	**69,679**
7.29 ,,	*34·3*	–	–	4	380	–	520	23	–	20	9	20	8	–	25	1820	100	1320	180	160	560	4	–	3,040	1180	–	40	–	**42,156**

Clione limacina in Plymouth Waters.

By

Marie V. Lebour, D.Sc.,

Naturalist at the Plymouth Laboratory.

With Plates I and II.

The pteropod *Clione limacina* (Phipps), now regarded as one of the tectibranchs, occurs at times in the neighbourhood of Plymouth. In 1930, however, it was extraordinarily abundant, especially in the summer, and was breeding freely. Although not specially recorded it has been seen singly or in small numbers in almost any month of the year, and for some years the eggs and larvæ (not recognised at the time as belonging to Clione) have been seen in summer. So far it is known from the British coasts in the North Sea and the west and extreme south-west of England. Its distribution, as known up to 1908, is given fully for the area of International Fisheries Investigations by Paulsen (1910). There it is shown that it is an Arctic-boreal species, spreading southwards nearly to the Bay of Biscay, which is about its southerly limit. It is known to occur in the northern and mid North Sea, but not in the southern North Sea, and at that time was only recorded from the Channel in the extreme west. It is also common in the Atlantic. Paulsen was led to infer that *Clione limacina* did not enter the North Sea from the Channel, but its prevalence sometimes at Plymouth shows that it can come a long way up the Channel and, as few samples are taken to the east and in the southern North Sea, this view should be carefully reconsidered. It was also thought that the presence of *Clione limacina* indicated Atlantic water, but as it may be found in almost any month and breeds at Plymouth in summer, and as its large numbers do not specially coincide with any influx of Atlantic water, it seems more reasonable to suppose that it is a permanent member of the Channel plankton.

Paulsen (*op. cit.*) thinks that the nets used are too small for Clione, but at Plymouth the larvæ may be found in the finest tow-nettings (180 strands to the inch). It is perhaps more probable that the small larvæ, if present, are not generally recognised. At Plymouth the larvæ have been found in the tow-nettings of any mesh, and the adults in medium and course tow-nettings and in the ring-trawl.

Clione limacina usually occurs with *Limacina retroversa*, on which it probably feeds, as the usual food in Arctic waters is *Limacina arctica*, a closely related species. It also feeds on other plankton organisms, being extremely voracious and armed with powerful hooks, strong radula, tentacles, and head-cones. It is itself economically important, for not only does it form an important food for whales in Arctic waters, but is also largely eaten by mackerel and herring. Hardy (1924) states that in the North Sea *Clione limacina*, although of much less importance than Limacina, was taken in fair numbers in the summer of both 1922 and 1923 and formed about 0·21 per cent of the total year's food of the herring.

M'Intosh (1898) has described the late larvæ in various stages from St. Andrews Bay, all of which agree well with the Plymouth specimens. He shows that the southern examples are much smaller than the Arctic forms. Paulsen (*op. cit.*) is of the opinion that the Arctic form and the more southerly form are two distinct races. The Plymouth specimens are mature at 4 or 5 mm., the largest reaching to 12 mm. ; even at 3 mm. they may contain quite large eggs. M'Intosh's largest specimen measured 12 mm. The Arctic form reaches 40–41 mm. In support of Paulsen's view it is found that there are many more teeth in the radula of the adult Arctic form. The usual formula is 14–1–14 or 13–1–13, the side teeth being less in the younger individuals and gradually increasing with age (Pelseneer, 1886). In the Plymouth form the formula is 4–1–4 or 3–1–3 for all adults examined, ranging from 4 to 10 mm. in length. In no case have more than 4 side teeth been found. Thus the adults of the southern form have a radula formula similar to the young of the Arctic form. Another difference is the colour, which is much less intense than in the Arctic form, the hermaphrodite organ being a pale pinkish yellow instead of the brilliant orange-red of the Arctic form. It appears thus that we have truly a southerly form of *Clione limacina* differing from the northern form in size, radula formula, and colour.

In the Arctic regions *Clione limacina* spawns in shallow water ; in the Atlantic it spawns in the open sea. So far the breeding habits of the southern form have never been described.

Clione limacina may occur at Plymouth in any month of the year. The largest specimens have been seen in February and March and in August. The greatest number were seen in the summer of 1930 when all through June until the middle of August they were abundant, adults, young, and eggs were to be found in the plankton both from the outside waters and inside the Sound. Mr. O. D. Hunt notes that it also occurred abundantly in the neighbourhood of the River Yealm, going up the river, and that they reached 10 mm. in length. They were not seen to breed except in the summer months and at this time nearly every individual from 3 mm. in length carried eggs. If one of these laid eggs in a bowl, more eggs were seen

inside, indicating more than one brood. Moreover, development takes place very quickly, and probably only a few months are needed for quite a large size to be attained. It is very likely that those eggs laid in the summer yield mature animals the following summer.

If placed in a bowl on the Laboratory bench the mature individuals will nearly always lay eggs overnight. From these eggs some of the larvæ were reared until the stage in which the shell was lost and the three circlets of cilia were formed.

The later larvæ of *Clione limacina* have often been described, but the younger larvæ which are provided with a shell are not so well known, although very young larvæ of other species of the Clionidæ have been briefly described (Krohn, 1860). From the work of Fol (1875) we know the general form of the spawn of various pteropods, which appears to be of the same character in all species, both thecate and naked. The eggs are minute and laid in a perfectly transparent gelatinous ribbon, which is sometimes free and sometimes entangled in any light hairy object which may be floating in the plankton. There seems to be no reason to think, as Paulsen (*op. cit.*) has suggested, that they are laid on the bottom, for the eggs of both Clione and Limacina frequently appear in the plankton at Plymouth. The eggs are placed in the gelatinous ribbon at regular intervals, arranged differently in the different species, and are themselves perfectly transparent and begin to develop when only a few hours old, emerging as larvæ, with or without a shell according to the species.

Clione limacina has been described many times, the chief works being those of Eschricht (1838), Souleyet (1852), Boas (1886), Wagner (1885), Pelseneer (1886) and Meisenheimer (1906). All these treat mainly of the purely Arctic form. M'Intosh's (*op. cit.*) description of the southern form from St. Andrews Bay shows the differences between the two.

The eggs of *Clione limacina* are laid in a clear oblong or square gelatinous mass, sometimes more elongated, the matrix being very elastic and catching in any light obstacle which may be floating about. A typical mass may measure about 1 mm. to 1·2 mm. across, the perfectly colourless eggs being scattered about irregularly, of an oval shape, and measuring about 0·09 mm. by 0·08 mm. (Plate I, Figs. 1 and 2). In two or three days the larvæ emerge. The newly-hatched larva has a thimble-shaped shell, usually slightly irregular, measure about 0·15 to 0·16 mm. in length and 0·11 mm. in breadth. The shell is very thin and perfectly transparent, slightly pointed at the end, with the mouth widening a little. The larva is fixed to the shell by longitudinal muscles reaching from near the tip to the mouth of the shell (Plate I, Figs. 3, 4, 5). There is a well-developed bi-lobed velum edged with large cilia and a ridge below edged with small cilia, the two forming a groove to the mouth. The whole animal, including

the velum, is capable of complete retraction into the shell. The beginning of the foot sticks out between the velar lobes and is ciliated all over, stomach, intestine, and liver are seen and there are two conspicuous otoliths. The larva grows rapidly ; two or three days after hatching the shell has increased in length to about 0·18 mm. and the margin of the shell is finely striated longitudinally (Plate I, Fig. 6). The velum projects far out of the shell when the animal is swimming. In the next few days the shell rapidly increases, very soon almost doubling its original length. It has now reached the maximum size (Plate I, Figs. 7–8). Spicules can be seen lining the stomach and in it remains of food. The larvæ in the bowls were given a pure culture of Nitzschia and the spermatozoids of Fucus, but the food inside the stomach was not usually recognisable. The dinoflagellate *Prorocentrum micans* was once seen in the œsophagus of a larva which had lost its shell. There is strong ciliation down the intestine, œsophagus, and round the mouth region. The shell now disappears. It was not possible to be certain whether it was cast off or absorbed, but it is probable that it was cast off, as empty shells were found in the bowl. There are now three circlets of cilia round the larva, one at the neck, just below the velum, one near the centre of the body, and one at the hind end (Plate I, Fig. 10 ; Plate II, Figs. 1–6). The whole body is covered with very fine cilia. Beginnings of the radula can now be seen and the tentacles and head-cones begin to form ; the foot shows a small hind lobe and the velum very soon disappears. The larvæ were reared in the finger-bowls until the disappearance of the velum when they were 17 and 18 days old and measured about 0·32 mm. in length. After this they died. They were still quite colourless and transparent. Larvæ were then collected from the plankton, the earlier stages corresponding exactly with those reared in the bowls. From this stage onwards the internal organs gradually move forward so that, as has been noted by many workers, in the adult they occupy only the fore part of the body, leaving the hind part for the developing ova. Larvæ from the plankton slightly older than those reared in the bowls and measuring 0·48 mm. in length had head-cones, tentacles and hooks well developed, the radula present, the wings beginning to form behind the foot ventrally (Plate I, Fig. 11). The liver and intestinal mass has begun to move forward. At about 0·9 mm. to 2 mm. the wings are still more developed and at 2 mm. they may project well beyond the sides of the body. Whilst they are developing the ring of cilia at the neck becomes irregular as it is interrupted ventrally by the foot and wings. At 2 mm. the intestinal mass lies almost entirely in front of the middle ring of cilia (Plate II, Figs. 1–6). At 2·8 mm. all the cilia may have disappeared and the wings are well developed, the penis showing at the right side. The last row of cilia may however persist even when the animal is breeding. In a specimen 3 mm. long all the organs are formed,

and it may now lay eggs. One specimen, 2·8 mm. long, had eggs nearly ripe occupying the hind end of the body (Plate II, Fig. 7) and one of 3 mm. laid eggs in the Laboratory. Fol (*op. cit.*) notes that a young *Clione aurantiaca* Fol. from Messina which still retained the last ring of cilia was full of eggs. Pelseneer (*op. cit.*) suggests that this is *Clione flavescens*. The animals have gradually been acquiring red and orange pigment in parts, and although the adult is fairly transparent it is much less so than in the young stages. The head end is bright orange-red, especially the head-cones and tentacles and round the mouth, also orange-red at the extreme hind end, the penis being pinkish yellow. The liver which surrounds the intestinal mass is dark brown and very conspicuous, the gonad sometimes pinkish yellow or sometimes colourless and the wings a pale pink.

In 1930 Clione was unusually abundant in the neighbourhood of Plymouth, but it had also been noticed in fair numbers, though not specially recorded, in the previous years. In 1931, however, it was not seen after January until late in August, when it was plentiful in the outside grounds. It is evidently irregular in its appearances. The following records from 1930 to September, 1931 (when it was last seen), are given, showing that it has been observed every month during one year :—

1930. *January, February, March, April, May*— in ring-trawl, from outside, fairly large specimens, especially in February. In tow-nettings occasionally both inside and outside Sound.

June. Abundant all through the month. Many adults and young, especially from outside. Eggs just seen in fine tow-net from W. of Eddystone on June 30th.

July. Eggs, young of all ages, and adults very plentiful, inside and outside. On July 17/18 one specimen of 4 mm. laid eggs during the night in a finger-bowl. Many more did the same all through July.

August. Many in early part outside and inside, eggs, young and adult, gradually dwindling, very few in latter part.

September. Very few. A few still breeding.

October 28th. One 1·9 mm. long, with hind circlet of cilia, from beyond Eddystone.

November 6th. One with 3 circlets of cilia, from outside. *November 17th*. Two with 3 circlets of cilia, 4 miles south of Penlee. *November 21st*. One larva with 3 circlets of cilia. Between New Grounds and Breakwater.

December 3rd. Three larvæ with 3 circlets of cilia, off Eddystone. *December 4th*. One with 3 circlets of cilia, off Rame. *December 18th*.

Three with 3 circlets of cilia, from Sound. *December 19th.* Two with 3 circlets of cilia, off Mewstone.

1931. *January 19th.* Two with 3 circlets of cilia, off Mewstone.

August 25th. A few mature specimens and one larva with 3 circlets of cilia, outside Sound. Some laid eggs in the Laboratory which hatched.

26th. About 150 mature specimens and many larvæ from Station E 2 between Eddystone and Ushant.

28th. A few adults, one larva with 3 circlets of cilia, near Eddystone. Two larvæ from inside the Sound.

September 3rd. Many adults and larvæ with 3 circlets, near Eddystone.

9th. A few adults and larvæ, near Eddystone.

16th. Two adults and a few late larvæ, near Eddystone.

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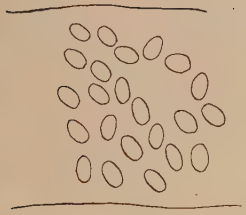
KEY TO FIGURES.

(Scale B is 6 times the scale of A.)

PLATE I. (Fig. 1 Scale A., 2—11 Scale B.) *Clione limacina.*

FIG. 1.—Ribbon of eggs, laid in finger-bowl, 30.6.30.
FIG. 2.—Egg a few hours old, 0·11 mm. long.
FIG. 3.—Empty shell of newly hatched larva, 0·14 mm. long.
FIG. 4.—Shell of newly hatched larva with animal retracted, 0·16 mm. long.
FIG. 5.—The same, side view, swimming.
FIG. 6.—Young larva, five days old, reared in Laboratory, 0·32 mm. long.
FIG. 7.—Empty shell of larva 12 days old, reared in Laboratory, 0·28 mm. long.
FIG. 8.—Larva 14 days old, reared in Laboratory, 0·38 mm. long.
FIG. 9.—Larva 16 days old, reared in Laboratory, having lost its shell, 0·35 mm. long.
FIG. 10.—Larva 18 days old, reared in Laboratory, having lost its velum, 0·32 mm. long.
FIG. 11.—Larva from plankton, 0·48 mm. long, side view.



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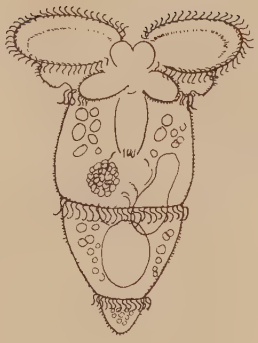

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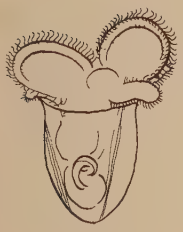

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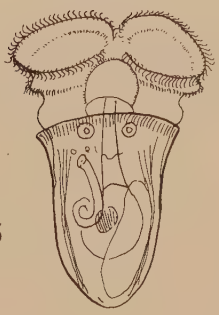

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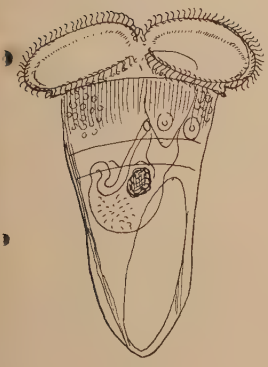

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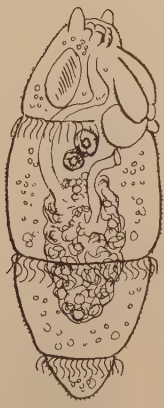

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PLATE II. (Scale A.) *Clione limacina* from plankton.

FIG. 1.—Larva 0·96 mm. long, side view.
FIG. 2.—Larva 1 mm. long.
FIG. 3.—Larva 1·44 mm. long, ventral view.
FIG. 4.—Larva 1·6 mm. long, side view.
FIG. 5.—Larva 2 mm. long, dorsal view.
FIG. 6.—Larva 2 mm. long, ventral view, older than Fig. 6.
FIG. 7.—Larva 2·8 mm. long, with developing eggs.
FIG. 8.—Head of adult showing radula protruded.

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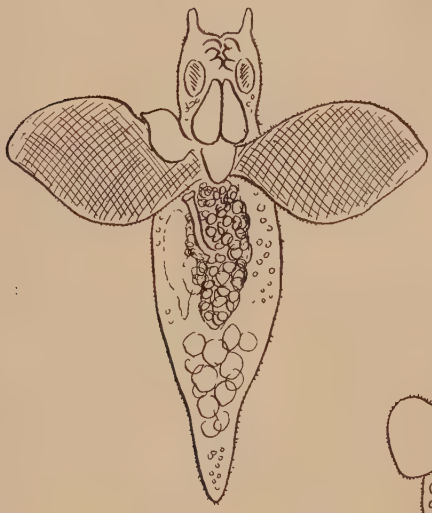

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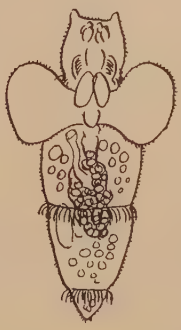

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8

The Larval Stages of *Nassarius reticulatus* and *Nassarius incrassatus.*

By

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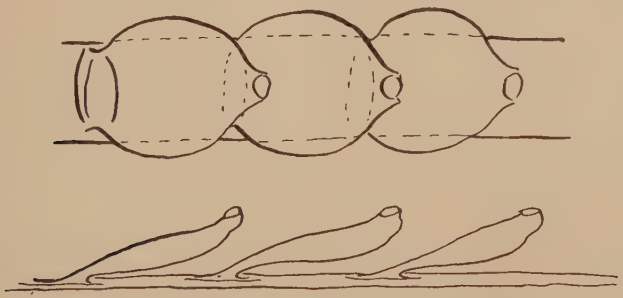
With 3 Figures in the Text and Plates I–V.

The two species of Nassarius (usually known as Nassa), *N. reticulatus* and *N. incrassatus*, are both common at Plymouth. *N. reticulatus*, the larger species, occurs from low water usually to a depth of a few fathoms, more rarely from the waters outside the Breakwater, *N. incrassatus* occurring between tide-marks and extending into deeper water, even to 46 fathoms.

The egg capsules of both are well known. It is necessary, however, to modify a general statement present in most popular mollusc and natural history works (for instance, Cooke, 1895, p. 125), quoted by Flatteley and Walton (1922, p. 272), that "*Nassa reticulata* lays egg capsules in shape like flattened pouches with a short stalk. . . . *Nassa incrassata* deposits solitary capsules which are shaped like rounded oil-flasks."



Text-Fig. 1.—Egg capsules of *Nassarius reticulatus* on Zostera.

In reality both lay egg capsules like flattened pouches, the general shape being much alike, but those of *Nassarius reticulatus* are nearly three times the height of those of *N. incrassatus ;* the pouch is flattened in one direction and almost round in the other, that of *N. reticulatus* being rather flatter than *N. incrassatus*. The adhesive base is broad in *N. reticulatus* and hardly to be called a stalk, whilst in *N. incrassatus* it

is narrower and more stalk-like. The mouth of the pouch is more pronounced in *N. incrassatus*. The capsules have a slanting position, the flat surface uppermost (Plate I and Text-figures 1 and 2). Both species may lay the capsules on Zostera, but this is usual in *N. reticulatus* and there are generally many in a row. There may, however, be only one, two or three, and occasionally they are laid in clusters of rows on hydroids or on Bryozoa. *N. incrassatus* usually lays the capsules irregularly in clusters on some rough surface, especially on hydroids or on Bryozoa, but occasionally they are to be found singly and sometimes on Zostera. When on Zostera they are more usually clustering in crevices near the roots, but as many as three in a row have been found on Zostera leaves. In support of this statement we find that Fischer (1892), who was the first to describe the capsules of what is almost certainly *N. incrassatus*,

Text-Fig. 2.—Egg capsules of *Nassarius incrassatus*.

states that they occurred two or three at a time on Zostera. His figure shows a broader base than in the Plymouth specimen, but the size (not over 2 mm.) and the fact that *N. incrassatus* was common where he collected the eggs make the identification almost certain.

From 50 to 100 eggs, sometimes less, sometimes more, are laid in the capsules. *N. incrassatus* usually lays from 50 to 80, rarely more, in each capsule, *N. reticulatus* usually upwards of 100. Usually all the eggs develop and there are no "nurse eggs," which are devoured by their neighbours, as is the case in certain other gastropods, notably *Buccinum undatum*, *Nucella* (=*Purpura*) *lapillus*, *Natica catena* and *Rissoa membranacea*. There is thus an enormous number of young hatched out and swimming about in the sea as veligers, both species hatching with well-formed shell and bilobed velum. Up to this stage the larvæ, especially those of *N. reticulatus*, are well known, and it is apparently usually supposed that they have a short free-swimming life and soon settle down to be like their parents.

Pelseneer (1911) gives an account of the eggs and larvæ of *N. reticulatus* up to the time of hatching, but he could not rear them longer than two or three days. He describes and figures the developing embryos and newly-hatched larvæ and his descriptions agree very well with the Plymouth specimens. He also figures the egg capsules and larval shell in Lankester's *Treatise of Zoology ; Mollusca*, 1906, pp. 130, 135, and the egg capsules again in his paper of 1926, in which he describes the egg-laying. Recently Ankel (1929) has investigated the laying of the egg capsules in *N. reticulatus* and given photographic illustrations of these. The following notes will show that both species have a long free-swimming period lasting for two months or more (at any rate under Laboratory conditions) and grow to a large size before losing the velum. For this

Text-Fig. 3.—Larvæ of *Nassarius incrassatus*.

reason they are a very important constituent of the plankton both inshore and outside, and they must form a large part of the food of the larger plankton-eating animals.

In late spring and summer a larval gastropod is always conspicuous in the Plymouth plankton, occurring in the water outside the Breakwater more commonly than any other mollusc (except the pteropod *Limacina retroversa* which may be present at the same time in very large numbers) and it is also common in the inshore plankton. The later stages of this larva, and it is these which commonly occur outside, show an enormously developed four-lobed velum. By keeping these in a plunger-jar it was found that they grew into *Nassarius incrassatus* (Plate IV, Fig. 8 ; Text-figure 3). Adults were then collected, eggs obtained and the larvæ reared to a certain stage. Eggs of *N. reticulatus* were obtained from the Zostera, hatched and reared until they began to crawl. This took two months. There is every indication that the free-swimming period is even longer in *N. incrassatus*.

It is now possible to give an account of the life-history of both these species from experiments and observations in the Laboratory and from freshly collected material from dredge, trawl and tow-net.

Methods.

Eggs of both species were collected whenever possible and plankton hauls investigated continually. The two species of Nassarius were placed in aquaria, aerated glass vessels, plunger-jars and Laboratory tanks.

Although later on some were found to be depositing eggs in the tanks, in none of these cases did *N. reticulatus* lay eggs in captivity, but *N. incrassatus* laid them easily in plunger-jars on the glass, on Zostera roots and on pieces of Bryozoa and hydroids. The eggs of *N. reticulatus* laid on Zostera were collected, the capsules removed from the Zostera and placed in a plunger-jar. These hatched out early and were kept in the same vessel for over two months without change of water. A pure culture of Nitzschia was added to the water, and this diatom, together with others which appeared naturally in the water, served as food for the larvæ. Unfortunately towards the end of the two months the flagellate Phæocystis appeared in the jar, and although this was eaten by the larvæ to a certain extent it soon became too dense and killed everything off. Some of the larvæ had, however, reached the crawling stage. From the first it could be seen that diatoms and other minute objects were in the stomachs of the larvæ, the contents always in a violent state of agitation owing to the ciliated lining of the stomach. Later on larger diatoms could be detected and the larvæ ate *Skeletonema costatum* and *Thalassiosira gravida* besides Nitzschia. These were the main forms growing in the jar and it was interesting to note that chains of Skeletonema and Thalassiosira were to be seen in the stomach and not only separate cells. That Skeletonema, which is one of the commonest diatoms, one of the easiest to culture, and one which is always penetrating into other cultures, should be a common food for a larval mollusc is an interesting and important fact. It is evident that diatoms form the chief food of these larvæ throughout their free-swimming life.

The larvæ of *N. incrassatus* were also fed in the same way, but they did not live so long and intermediate stages were collected from the plankton. In all cases when examined for food the natural food of both species in the free-swimming stage was essentially diatom material. The late larvæ of *N. incrassatus* were put into a plunger-jar provided with diatoms. As they grew and began to crawl, small mollusc larvæ from the plankton, chiefly bivalves, were introduced and evidently served well as food for those which had lost the velum. A stage exists in both species in which the velum is still retained although the foot is well developed. The mollusc can thus crawl or swim at will. It is apparently still a plankton feeder for some time and continues to eat diatoms, but when the velum begins to disappear, and afterwards, it is apparently carnivorous. Small bivalves, especially Anomia, settled down on the glass sides of the plunger-

jar and these were eaten by the young Nassarius, some of which were actually seen to attack them. In this way *N. incrassatus* was reared to the adult stage.

The water for rearing was taken from outside the Breakwater where there were many diatoms, and these diatoms naturally present were eaten quite as much as the Nitzschia which was introduced. In all cases the food was chiefly Skeletonema, Thalassiosira and Nitzschia, always mixed with small indistinguishable particles, probably both organic and inorganic.

Characters of the Two Species.

The egg capsules are a light horn colour, transparent, and the eggs visible through the wall of the capsule (Plate I). About a month is the usual time taken from the deposition of the eggs to the time of hatching. This is the time given by Pelseneer, and it is much the same in the Laboratory, the eggs of *N. reticulatus* from very early capsules hatching in from three to four weeks, those of *N. incrassatus* newly laid in the Laboratory hatching in twenty-three to thirty days. The ages of the larvæ when known and the sizes given are all under the ordinary Laboratory conditions of temperature. A comparison with those from the plankton shows much the same state of development at the same size. Some time before hatching the larvæ are moving about as veligers inside the capsule, with the shell already formed, much yolk being inside each larva, and when all the yolk is absorbed the larva hatches, escaping from the top of the capsule where a thin covering is broken through.

The newly hatched larva (Plate II, Figs. 1, 2; Plate III, Fig. 1; Plate IV, Figs. 1, 2) has a smooth unsculptured shell, very transparent, consisting of one whorl. The outer lip is slightly drawn out at the centre, curving inwards so that two hollows are formed to support the velum. This projection grows into a conspicuous tooth bent inwards and the hollows deepen as the velum grows (Plate III, Fig. 2; Plate V, Figs. 3, 7). The animal has a bilobed velum, rather longer across than the greatest breadth of the shell, with the usual ciliated margin and ciliated ridge below forming a groove to the mouth. The general method of feeding in a veliger is for the food to be collected whilst the animal is swimming. This is done by means of the velum. Round the margin of the velum is a thick rim edged with large cilia. On the underside and somewhat internal to the margin is another smaller ridge edged with smaller cilia. These two ridges form a groove in which the food is collected and brought to the mouth (Plate V, Fig. 6). The mouth is also ciliated and the united workings of the cilia on the velum and round and inside the mouth bring a constant supply of small food to the animal. Mucus appears to be secreted in the groove and if the particles are not suitable the animal

swims away from them, leaving them behind entangled in a stream of mucus. However large and elaborate the velum may be, the process of feeding seems to be the same. As the foot grows and crawling begins the radula has been forming and soon a different method of feeding may come into use, until finally the velar method is abandoned and the ordinary adult carnivorous method substituted.

At first the paired eyes are at the base of very short tentacles. Sometimes, as Pelseneer (1911) points out, but not always, only one tentacle is developed, the other not appearing till later. There are paired otoliths at the base of the foot. The ventral mouth just below the velum, œsophagus, stomach, intestine and anus are all visible, the liver lobes occupying the apex of the shell, the kidney is present and the heart beating. The stomach is ciliated and food material always moving about inside. The rectum and anus are on the right side (Plate III, Fig. 5 ; Plate V, Fig. 5). The veliger moves quickly by means of the long cilia on the velum which is fully expanded. Later the flapping of the lobes helps in the movement.

The larva grows quickly, but has a long free-swimming period. After a few weeks the velum becomes four-lobed and the colour, if not already present, is accentuated in certain regions, the shell begins to be spiral and the shell siphon or canal is formed (Plate II, Figs. 2 -11 ; Plate III, Figs. 3–5 ; Plate IV, Figs. 3–8 ; Plate V, Figs. 2–5). The velum may remain only slightly four-lobed (*N. reticulatus*, Plate II, Figs. 8–9), or the lobes may grow out to an enormous length (*N. incrassatus*, Plate IV, Fig. 8 ; Text-Fig. 3). The foot, situated behind the velum, is ciliated on the whole of its surface ; at first a very small projection narrowing behind, but always provided with an operculum, it soon lengthens and a few large cilia project at its hind end. In later stages the front of the foot grows forward into a flexible process, still ciliated (Plate II, Figs. 7–9 ; Plate IV, Figs. 7–8). The tentacles grow longer and are also ciliated, the tips having a few large cilia and the whole surface covered by minute cilia. Pigment appears on the base of the foot and round the mouth. In *N. reticulatus* there is from the first a border of brown pigment round the velum, in *N. incrassatus* the velum is at first colourless, then brown pigment appears and gradually concentrate with a large spot at each corner of the four lobes. After some weeks the shell has from three to four whorls and the canal (the shell siphon) is fully formed, containing the siphon proper. When the animal can crawl there are from three to four whorls, the larval shell mouth has no longer the projecting tooth but the margin is simple with a slightly crenulated edge (Plate II, Figs. 12–14 ; Plate V, Figs. 8–9). The head end projects slightly and the front of the foot is provided with two horns. The foot itself is elongated and the siphon very long. Ribs may begin to appear on the shell. The animal is

now outwardly like the adult except for the sculpture on the shell, and probably attains maturity in a year or less.

Nassarius reticulatus (L.).

(Plates I, Figs. 1–2 ; II and III : Text-Fig. 1.)

The eggs are laid in spring and summer, usually from March to August, more rarely in autumn and winter, in the inshore waters ; occasionally further out in deeper water. Records of eggs show that they may be laid every month, except October and November (*Plymouth Marine Fauna*, new edition, 1931). Probably a few eggs could be found in any month of the year. Breeding is usually finished by the end of September, but three capsules were found on Zostera from Cawsand Bay, 28.1.31, perhaps the earliest of the season. The best localities for the egg capsules are the Zostera beds in Cawsand Bay and off Drake's Island. One large mass from outside waters in June, 1931, consisted of many clusters of rows laid in a tangle of Antennularia and Sertularella.

The capsule measures about 4·8 to 5 mm. in height and about 4 mm. across the widest part. The newly laid egg measures about 0·16 mm. across, but grows rapidly and is much bigger as the embryo develops. The newly hatched larva measures about 0·28 mm. to 0·30 mm. across at its widest part, which is across the body whorl to the outer lip. The velum measures about 0·29 to 0·32 mm. across and has a continuous reddish brown border just inside the margin. The outer lip of the shell is conspicuous in its projecting centre curving inwards, forming almost immediately the characteristic tooth (Plate II, Figs. 1, 2 ; Plate III, Figs. 1, 2, 3). The shell and velum both grow quickly, the shell at five days old measuring about 0·32 mm. across, the velum 0·45 mm. across. The tentacles are well developed and there is a small amount of brown pigment on the ventral surface of the foot. At about three weeks old the shell measures about 0·4 mm. to 0·5 mm. across, the velum being about 0·48 to 0·55 mm. across. The outer lip is now drawn out posteriorly, forming the canal or shell siphon, and the spire begins to project. The foot is perceptibly longer and the dark pigment round the mouth is conspicuous. The larva now begins to show an alteration in the velum, and when about twenty-five days old there is an indentation at each side, the the first indication of the four lobes, and the width across grows larger. The animal now looks like a butterfly with outspread wings. The velum in *N. reticulatus*, however, is never so large, nor the lobes so long as in *N. incrassatus*. When about five weeks old the shell has from two to three whorls and measures about 0·56 mm. across, the velum being about 1·3 mm. across. The reddish-brown border to the velum is very conspicuous throughout the larval life. At about six weeks there are three

whorls to the shell and the velum, sometimes larger anteriorly, may measure about 2 mm. across. The foot has now grown out into a squarish lobe in front and there is much brown pigment. The tentacles are long. The size may vary and a rather later stage may be smaller than a younger one, as in Figs. 8 and 9 of Plate II. After this the anterior lobe of the foot expands into two horns and the animal is able to crawl. It may now measure 0·72 mm. to 0·8 mm. across from the outer lip margin to the body whorl; the outer tooth-like process has disappeared and the edge slightly crenulated (Plate II, Figs. 10–14). There is a long siphon, the foot projects some way beyond the spire when crawling and the tentacles are like the adult. It now crawls or swims at will, but the velum soon dwindles and then probably disappears altogether in a few weeks. At the first crawling stage *N. reticulatus* (about 0·8 mm. long) is decidedly smaller than *N. incrassatus* (about 1 to 1·2 mm.), although the latter species is so much smaller when adult.

All the stages are usually to be found in the inshore plankton in spring and summer and are seldom seen outside. They are exceedingly common inside the Sound.

Nassarius incrassatus (Ström.).

(Plate I, Figs. 3–4; Plates IV and V: Text-Figs. 2–3.)

The *Plymouth Marine Fauna*, new edition, 1931, records a mass of the egg capsules taken when the animal was actually depositing them on tufts of Antennularia, 25.1.09. Other records show that eggs were found every month except July. It is probable that a few may be found any month of the year, but the larvæ are much the commonest in spring and summer and are rarely seen in the autumn except when very young. Several adults were collected and placed in a plunger-jar in October and November, 1930, and on the 5th and 6th of November some capsules with eggs nearly ready to hatch were found in the jar attached to a mass of Cellaria on which were some eggs of *Parasepia elegans*. There were also capsules on the Parasepia eggs. Later in November and through December, January and February capsules were found on the sides of the glass jar, again on Cellaria and on pieces of Sertularella placed there for the purpose, and, later, from February to April, they were laid in clusters in crevices at the roots of Zostera floating in the jar. Very rarely they were laid on the Zostera leaves. Egg capsules were also found through May and June. All through June, July and August the late larvæ were abundant in the inside and outside plankton, and in winter, spring, summer, and more rarely in autumn the early larvæ could be found in the inshore waters. The younger forms are seldom seen far out. It is probable that most of the large larvæ come from inshore where the adults are so plentiful and they are so strong and large that they survive

when the smaller larvæ would perish. The length of larval life and their propensity for being carried out to sea would account for the very wide distribution and large numbers of this common species.

The egg capsules measure about 1·5 mm. to 2 mm. in height, the greatest breadth being rather less than the height. The eggs when newly laid measure 0·16 mm. across, which is the same size as those of *N. reticulatus*, although the newly hatched larvæ are smaller than in that species. The newly hatched larvæ are at first like *N. reticulatus*, but without any pigment on the velum. The shell measures 0·18 mm. to 0·2 mm. across and the velum about 0·2 mm. to 0·24 mm. across. At first growth and development take place in a similar way to *N. reticulatus*, but the pigment on the velum only appears after a few days.

Although the early larvæ are at first smaller than *N. reticulatus* at the same stage, yet in the later stages they are larger and the velum grows out into four very long lobes.

It was unfortunately not possible to rear these larvæ for longer than three weeks, but intermediate stages were collected from the plankton and the later stages were reared in a plunger-jar to the adult. Three of these which were placed in the jar in August, 1930, when they were free-swimming veligers, are now fully grown (July, 1931).

In about fourteen days the larvæ reared from the egg measured about 0·32 mm. across. Pigment may or may not be present on the velum at this stage, but at about three weeks old there are irregular masses of brown pigment which soon concentrate to form a large spot at each corner. The velum also begins to be four-lobed and shaped much like that of *N. reticulatus* at a slightly older stage, and when the shell is about 0·5 mm. across the lobes are much more divided than in the latest stage of *N. reticulatus*, at 0·8 mm. the lobes of the velum are about at their longest and each lobe is quite twice as long as the shell (Plate IV, Fig. 8). When the shell is about 1·2 mm. the animal can either crawl or swim and at about 1·5 mm. the velum is lost. It is probable that the larva of *N. incrassatus* remains in the free-swimming stage even longer than *N. reticulatus*, which takes two months under Laboratory conditions to reach the crawling stage.

These late veligers collected from the plankton were watched in the plunger jars. Here they could be seen swimming. Sometimes they remained still, the velum outspread upwards, the shell hanging down, or else the velar lobes were flapping and the movement was like that of the wings of a pteropod (Text-Fig. 3). When the lobes are spread out the animal is moving very slowly, and it is then that the food is directed into the mouth.

We have now ascertained the life-histories of both the Plymouth

species of Nassarius which can be recognised in all stages in the plankton and distinguished from one another and from other larval gastropods found with them. The unsculptured shell and the prominent tooth on the outer lip, which is apparent almost directly, distinguish the Nassarius larvæ from others of the same size. Later on the half-grown larvæ of *N. incrassatus* have a four-lobed velum with four spots much like a commonly occurring larva belonging to Natica (probably *N. catena*). The Natica, however, has no shell siphon and is always to be distinguished.

The two Nassarius larvæ are to be recognised in the following ways :—

N. reticulatus.	*N. incrassatus.*
All its larval life has a band of brownish-red pigment round the inside margin of the velum.	At first colourless, then faint scattered brown pigment forming an irregular border, later on concentrated in four large spots, one at each corner of the velum.
Velum at first bilobed, then four-lobed, but lobes never long.	Velum at first bilobed, gradually becoming four-lobed, the lobes in late stages very long.
Newly hatched larva 0·28 mm. to 3 mm. across shell.	Newly hatched larva 0·18 mm. to 0·2 mm. across shell.
First crawling stage about 0·75 to 0·8 mm.	First crawling stage about 1·2 mm. Probably more than two months from hatching to first crawling stage.
Two months under Laboratory conditions from hatching to first crawling stage.	

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KEY TO FIGURES.

Scale B is six times the scale of A.

PLATE I.—Egg capsules of *Nassarius reticulatus* and *N. incrassatus*. Scale A.

Fig. 1.—*N. reticulatus*, 0·48 mm. high.
Fig. 2.—The same, side view.
Fig. 3.—*N. incrassatus*, 2 mm. high.
Fig. 4.—The same, side view.

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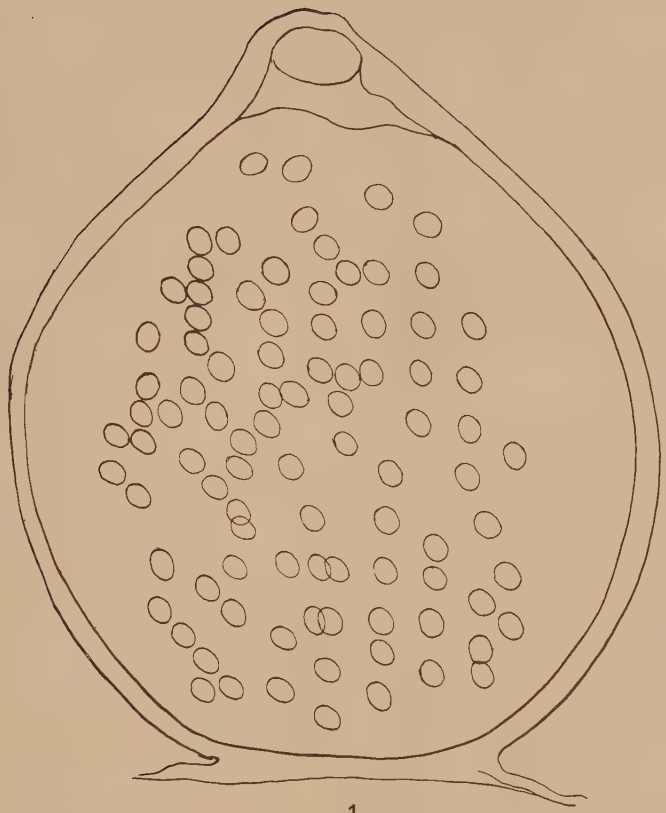

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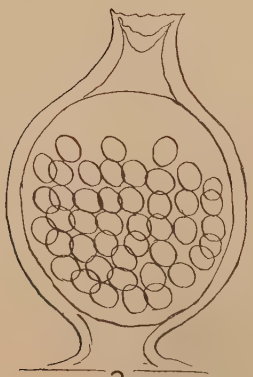

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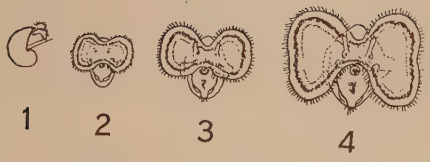
PLATE II.—Larvæ of *Nassarius reticulatus* reared from egg. Scale A.

Figs. 1–2.—Newly hatched, shell 0·25 mm. across body whorl.
Fig. 3.—7 days old, 0·32 mm.
Fig. 4.—19 days old, 0·45 mm.
Fig. 5.—25 days old, 0·56 mm.
Fig. 6.—32 days old, 0·64 mm.
Fig. 7.—35 days old, 0·7 mm.
Fig. 8.—40 days old, 0·75 mm.
Fig. 9.—48 days old, 0·75 mm.
Figs. 10, 11.—The same swimming in different positions.
Figs. 12–14. Crawling stages, 50 days to two months old.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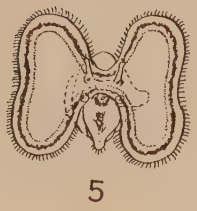
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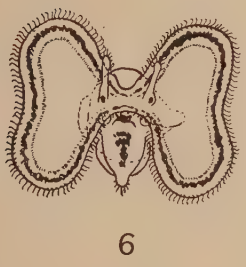
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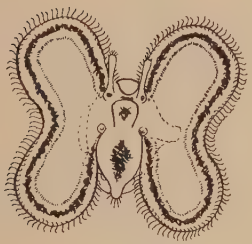
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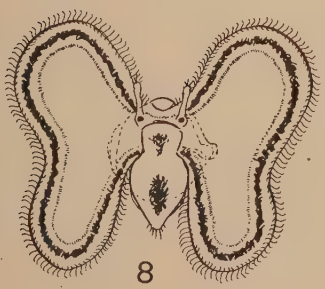
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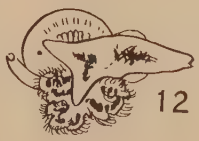
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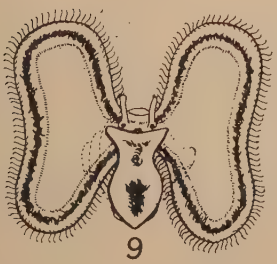
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PLATE III.—Larvæ of *N. reticulatus* reared from egg. Scale B.

Fig. 1.—Shell of newly hatched larvæ.
Fig. 2.—Shell of larva 5 days old.
Fig. 3.—Larva 3 days old.
Figs. 4–5.—Larva 19 days old.

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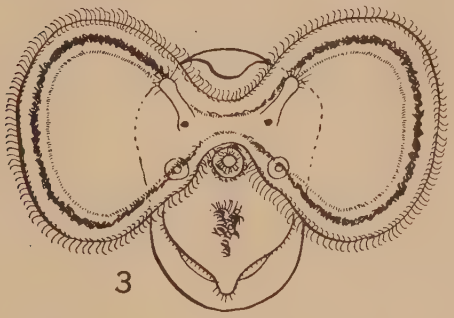

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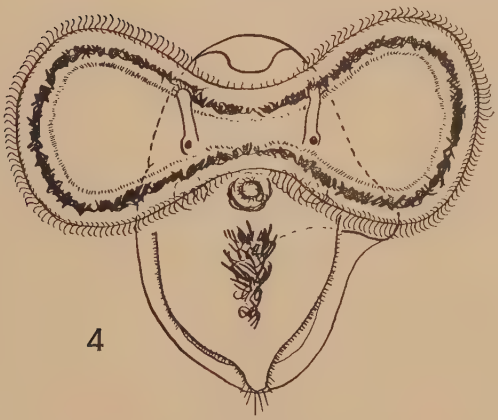

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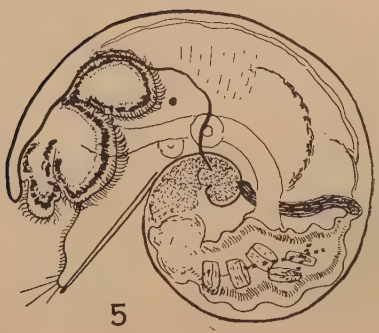

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PLATE IV.—Larvæ of *Nassarius incrassatus*. Scale A.
(1–3 from egg, 4–11 from plankton.)

Figs. 1–2.—Newly hatched larva, 0·18 mm. across body whorl.

Fig. 3.—14 days old, 0·32 mm.

Figs. 4–7.—Larvæ from plankton, the ages being probably from 3 weeks to 3 months or more.

Fig. 4.—0·35 mm. across.

Fig. 5.—0·44 mm.

Figs. 6–7.—0·55 mm.

Fig. 8.—0·8 mm.

Fig. 9.—Shell of Fig. 7.

Figs. 10–11.—Shells of crawling stage.

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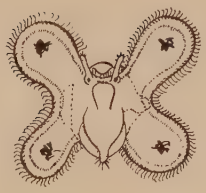
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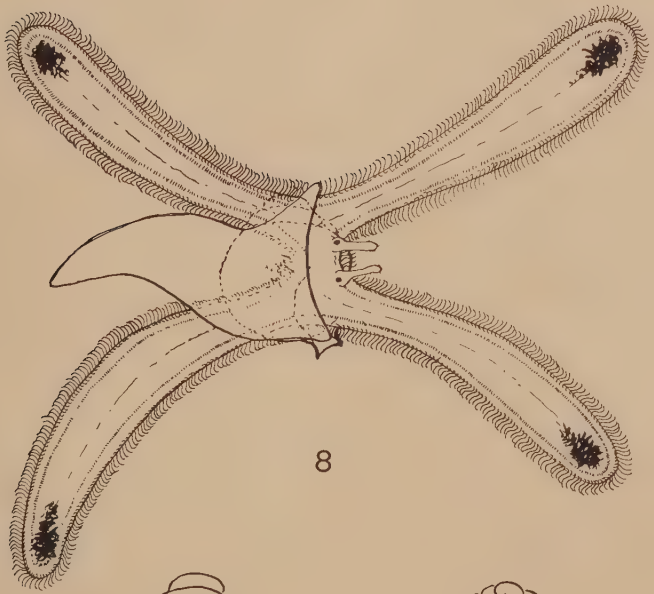
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PLATE V.—Larvæ of *N. incrassatus* (1–6 Scale B, 8 and 9 drawn on a smaller scale).

Fig. 1.—Larva still in egg capsule nearly ready to hatch.

Fig. 2.—3 days old.

Figs. 3–5.—2 weeks old.

Figs. 6–7.—Ca. 3 weeks old.

Fig. 8.—Larva crawling; but still retaining velum shell 1·2 mm. long.

Fig. 9.—Larva losing velum, shell 1·5 mm. long.

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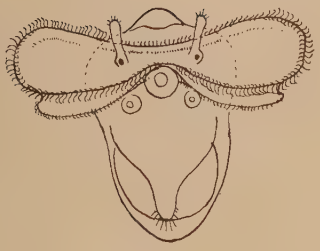

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The Larval Stages of *Trivia europea.*

By

Marie V. Lebour, D.Sc.,

Naturalist at the Plymouth Laboratory.

With One Text-figure and Plates I–IV.

In 1926 Pelseneer discovered the eggs of *Trivia europea* on the French coast. These he found were laid in capsules embedded in the compound ascidian *Polyclinum luteum*, the mollusc biting a hole and laying its eggs in a vase-like capsule completely enveloped in the ascidian except for the wide lip of the vase which projects freely and can be easily seen with the naked eye (Pelseneer, 1926, Figs. 11–12). He also hatched some larvæ from these eggs which were very interesting, as they possessed an accessory shell somewhat similar in shape to the Echinospira of *Lamellaria perspicua*. He did not obtain the eggs directly from the Trivia and was not able to keep the larvæ alive more than a few days. In his paper he expresses the hope that some worker will confirm his observations experimentally. This I am now able to do, having procured the eggs actually laid by the Trivia, hatched them out and kept them alive for more than a week, afterwards collecting the larvæ from the plankton from stages which exactly corresponded with those from the egg up to late stages which metamorphosed into young Trivia, and young stages of Trivia feeding on compound ascidians which corresponded with the metamorphosed forms.

Trivia europea is common at Plymouth between tide-marks and in a few fathoms of water; occasionally further out in deeper water. Records are available from the shore to the region beyond the Eddystone.

In order to obtain the eggs several adults were collected and placed in a plunger-jar. They were given various compound ascidians to eat and they ate largely of *Diplosoma listerianum* var. *gelatinosum* (=*Leptoclinum gelatinosum*). This ascidian grows well on the glass sides of the Laboratory tanks and is therefore a very convenient food. The Trivia also ate various forms of Botryllus, Botrylloides, and *Trididemnum tenerum*. Although placed in the plunger-jar in winter and spring and eating well they laid no eggs until June. On June 1st, 1931, several egg capsules were laid in an orange mass of Botryllus placed in the jar the night before (Text-Figure 1). These egg capsules were exactly similar to those found

by Pelseneer. Unfortunately the Botryllus died and also the eggs. On June 5th another capsule was laid in a small aerated glass vessel in which two Trivia and an orange Botryllus had been placed overnight. This Botryllus and eggs also died. During the night of June 14/15th two capsules were laid in a new lot of Botryllus in the plunger-jar. This was pale yellow and only a small piece growing on an old stalk of Eunicella. It was still living on June 16th, and between June 16th and 23rd there were six capsules in this piece of Botryllus. Some of these died, but on June 29th two capsules contained active embryos, probably those laid on the 14/15th. The Botryllus with the two capsules containing active embryos was then placed in a fresh plunger-jar and the young from one

Text-Fig. 1.—Egg capsules of Trivia in Botryllus.

capsule hatched on July 6th, those from the other capsule died. On June 29th two egg capsules were laid in *Tridemnum tenerum*, but neither the ascidian nor the eggs lived. Ascidians of an orange or yellow colour were always chosen for the eggs, probably because the eggs were themselves a bright orange and were thus protected. The larvæ from the capsules in Botryllus seemed to have hatched prematurely, for they still retained a large amount of yolk, the accessory shell was not completely formed and the true shell only indicated. The velum was present, however, and the larvæ were swimming about. In a few days they resembled the newly hatched young figured by Pelseneer (*op. cit.*, Fig. 16).

On the whole the Trivia seemed to prefer Diplosoma to eat and Botryllus to lay eggs in. They are usually found in the neighbourhood of Botryllus when collected between tide-marks, or they may be on Diplosoma. It is easy to watch them eating the ascidians with the long proboscis outstretched to its greatest length and the food rapidly entering the mouth (Plate IV, Figs. 5–6).

Although some of the Trivia have been kept for several months no more eggs have been laid since June. They must, however, breed in most months, for in any month of the year the larvæ both young and old may

be found in the plankton, perhaps more plentifully in spring and summer. They are quite common and usually to be found in the tow-nets from inside the Sound or a little way beyond, more rarely from deeper water.

The egg capsules measure about 4·8 mm. in height and contain several hundred eggs, each when newly hatched measuring 0·16 mm. across (Plate I, Fig. 1). The eggs are a bright orange, the capsules colourless and transparent, very thin where they are imbedded in the ascidian, but the lip is thickened where it projects and the eggs are covered over securely. The embryos when hatching break through the mouth-covering and swim away. Those hatched in the plunger-jar had a small bi-lobed velum, eyes on rudimentary tentacles with a projecting lobe in front, conspicuous larval kidneys (so called) just behind the velum, a mouth ventrally, otoliths at the base of the foot which is short and already provided with an operculum, the accessory shell forming and the true shell barely perceptible. Nearly all the body is full of orange yolk (Plate II, Fig. 1). The accessory shell measures about 0·18 mm. across. The unnatural condition may account for them being hatched prematurely. The plunger-jar in which the eggs were laid and hatched were on a Laboratory table under ordinary Laboratory conditions and temperature and the water was not changed at all. A pure culture of Nitzschia was given to the larvæ to eat and those of nearly a week old seemed to be feeding, but they did not live more than ten days.

The yolk disappears when the shell is about 0·24 mm. across, and the stomach, œsophagus, and intestine are developed. The animal is very dark, the liver being dark yellowish brown, the stomach, intestine, and œsophagus a dark purplish brown. These colours are retained throughout the larval life and are very characteristic (Plate I, Figs. 2–20 ; Plate II, Figs. 2–5). Later the outer margin of the velum has a very thin brown border. At 0·36 mm. across the accessory shell the true shell measures 0·16 mm. across. The velum is about 0·4 mm. across and very slightly indented at the centre of each side. The so called larval kidneys are still recognisable. At this stage the larvæ may be found in the plankton and are exactly like those which were reared from the egg (Plate I, Figs. 3, 4 ; Plate II, Fig. 4). They agree very well with Pelseneer's newly hatched larvæ. From this stage onwards larvæ of all sizes may be found in the plankton. The so-called larval kidneys now disappear and the true kidneys are formed, but the alimentary canal changes little. The velum grows large, the faint brown border appears in the later stages (Plate I, Figs. 17, 20), the front lobes are rather longer than the hind lobes. The velum has the usual structure, having a ridge with long cilia on the margin and an inner ridge of smaller cilia below, the two forming a groove to the mouth. The foot develops two large lateral lobes which help to support a very large spiral operculum (Plate III, Figs. 1, 2). When the accessory

shell is about 1·25 mm. across there is an anterior lobe to the foot which hides the mouth. The foot in the larvæ usually has scattered brown pigment on the sole. The tentacles are long and the mantle begins to project from the true shell. A gill is beginning to form at this stage (Plate I, Figs. 18–20). The accessory shell is perfectly clear and unsculptured, the only structure being a few lines of growth. The outer lip projects slightly. The true shell lies within the accessory shell and its whorls correspond in those of the accessory shell. This is an important difference which distinguishes it at once from the Echinospira of *Lamellaria perspicua* in which the whorls of the accessory (Echinospira) shell do not correspond with those of the true shell, the latter being placed excentrically. Another Echinospira occurring at Plymouth, probably belonging to Velutina, is like Lamellaria in this respect (Plate III, Figs. 8 9 ; Plate IV, Figs. 1, 2, 3). This unidentified Echinospira in its younger stages is like Trivia in many ways, including the shape of the velum. These facts emphasise the probable relationship of Trivia and the Lamellariidæ which has been suggested by Pelseneer (*op. cit.*).

At about 1·25 to 1·4 mm. across the accessory shell Trivia begins to metamorphose. This is done in quite a different way from Lamellaria, for whereas in Lamellaria the Echinospira shell is cast off as a crumpled skin, the mantle immediately surrounding and covering the true shell, in Trivia the accessory shell remains and the mantle surrounds and covers it (Plate III, Figs. 3–4). It is then probably dissolved. The foot loses its lateral lobes and operculum, a short siphon is formed and the animal crawls (Plate III, Figs. 5–7). As the mantle comes up round the shell, small protuberances with stalks embedded in the mantle are conspicuous. These look like soft nails with yellow heads placed irregularly round the base of the mantle and become the mantle-papillæ of the adult (Plate III, Figs. 5, 7 and 13). The shell soon becomes oval in shape and the body whorl elongated along the main axis. It is pearly white and perfectly smooth (Plate III, Figs. 10 and 11). This young shell is well known. Vayssière (1923) gives a figure of it. The siphon elongates and the mantle gradually covers the shell when the animal is active, but the whole animal may be completely withdrawn into the shell. Young individuals can be found crawling on compound ascidians in the Sound (Plate III, Figs. 12–13). The body and mantle can be variously coloured, the prevailing colours being cream, yellow, orange or brownish purple with small or large spots. Pale yellow with purple spots is a very usual combination, or brownish with orange spots, or the whole may be bright orange with various dark spots. The papillæ which are arranged chiefly on the sides of the lower surface of the mantle are yellow or orange, and there may be yellow or orange spots on the tentacles.

In the plunger-jars the larvæ from the plankton grow quickly and also

grow well after metamorphosis. It seems probably that it takes about a month or five weeks under Laboratory conditions from hatching to metamorphosis, a few weeks sufficing for the animal to obtain the elongated adolescent shell covered by the mantle. The adults live well in a plunger-jar. Some have been in the Laboratory for nine months with only two changes of water and are still alive (September).

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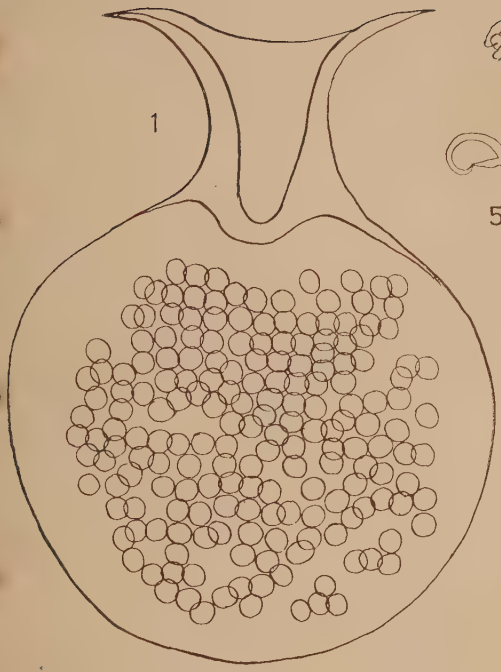
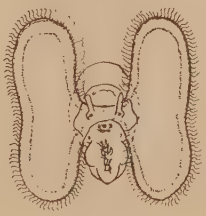
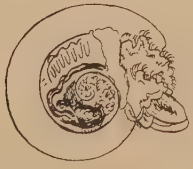
Vayssière, A. 1923. Recherches zoologiques et anatomiques sur les mollusques de la famille des Cypræidés. Ann. du Musée Nat. de Marseille. Zool., XVIII, pp. 1–1132.

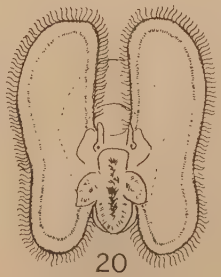
PLATE I. (Scale A.)

Fig. 1.—Egg capsule from Botryllus, 4·8 mm. high.
Fig. 2.—Young from egg, 5 days old, 0·35 mm. across shell.
Fig. 3.—Young from egg, 7 days old, 0·40 mm. across shell.
Figs. 4–20.—Young from plankton.
Fig. 4.—0·4 mm. across shell.
Figs. 5, 6, 7.—0·48 mm. across shell.
Figs. 8, 9.—0·56 mm. across shell.
Figs. 10, 11.—0·64 mm. across shell.
Fig. 12.—0·70 mm. across shell.
Figs. 13, 14.—0·80 mm. across shell.
Figs. 15, 16, 17.—0·96 mm. across shell.
Figs. 18, 19, 20.—1·25 mm. across shell.









Eggs and Larvæ of *Trivia europea*.

PLATE II. (Scale B, six times the size of A.)

Fig. 1.—Young newly hatched from egg, 0·18 mm. across shell.
Fig. 2.—Young from egg, 0·24 mm. across shell.
Fig. 3.—Young from egg, 5 days old, 0·35 mm. across shell.
Fig. 4.—Young from egg, 7 days old, 0·36 mm. across shell.
Fig. 5.—Young from plankton, 0·64 mm. across shell.

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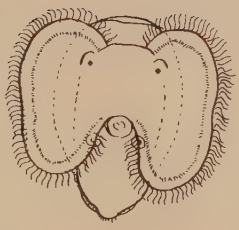

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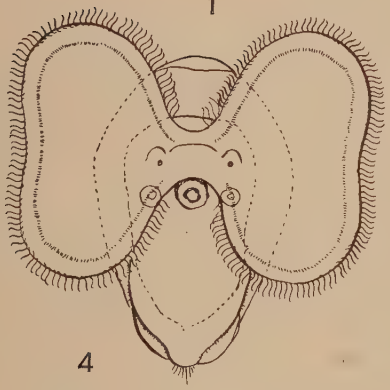

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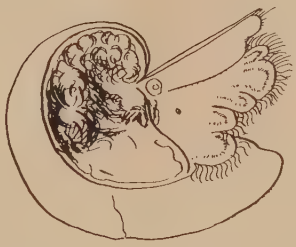

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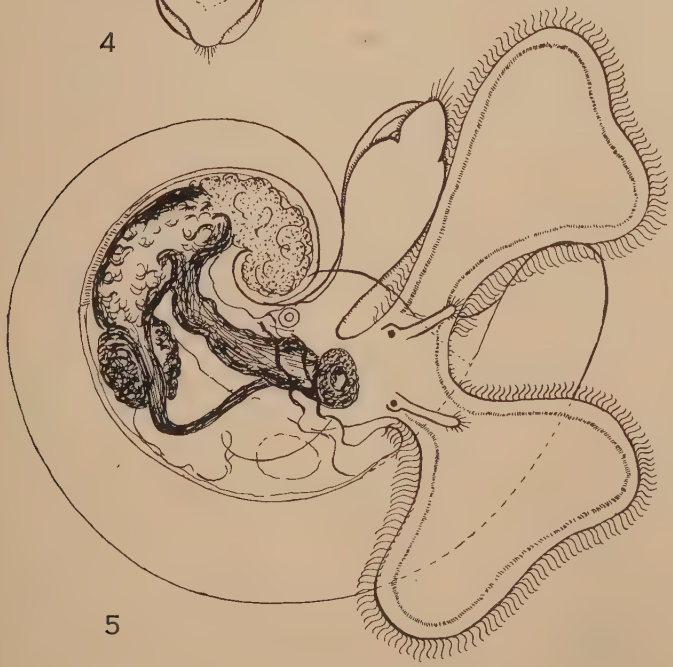

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Larvæ of *Trivia europea*.

PLATE III. (1–4 and 8, 9, Scale A, the others on a smaller scale.)

Fig. 1.—Operculum of young, 0·64 mm. across shell.
Fig. 2.—Operculum of young, ready to metamorphose.
Fig. 3.—Young from plankton, 1·44 mm. across shell.
Fig. 4.—The same metamorphosing.
Figs. 5–7.—The same after metamorphosis.
Figs. 8, 9.—Empty shell, 1·4 mm. across.
Figs. 10, 11.—Shell of older individual grown from young in plunger-jar, 4 mm. long.
Figs. 12, 13.—Young found crawling on Diplosoma, from Sound, shell 3·5 mm. long.

Larvæ and young of *Trivia europea*.

PLATE IV. (1–3, Scale A, others on smaller scale.)

Fig. 1.—Young Trivia ready to metamorphose, 1·44 mm. across shell.

Fig. 2.—Unknown Echinospira, probably Velutina, ready to metamorphose, 1·92 mm. across shell.

Fig. 3.—Echinospira of *Lamellaria perspicua* ready to metamorphose, 2·24 mm. across shell.

Fig. 4.—Adult Trivia.

Figs. 5, 6.—Adult Trivia eating Diplosoma.

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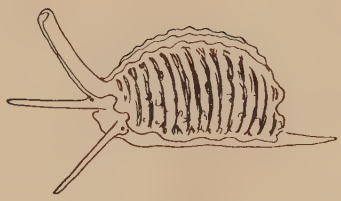

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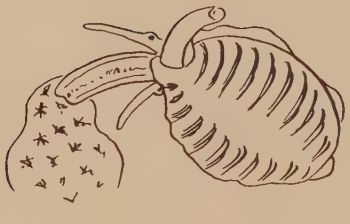

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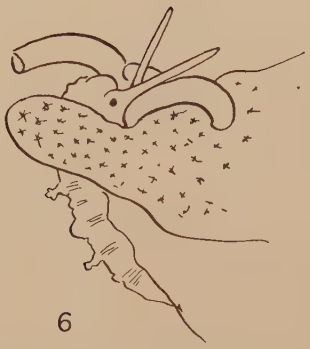

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Late larvæ of Trivia, Velutina (?) and Lamellaria. Adult Trivia.

Note on a Marine Labyrinthula.

By

Margaret W. Jepps,

Lecturer in Zoology, Glasgow University.

With 6 Figures in the Text.

Plasmodia of Labyrinthula almost invariably appear in the diatom cultures and other small marine aquaria which I have kept during the last several years for a variety of purposes in Glasgow. These aquaria are stocked with material from Millport, I. of Cumbrae, and I think it quite certain that the Labyrinthula is imported thence.

This amazing creature has attracted interest ever since it was first described by Cienkowski in 1867, and good figures of it have been published by Cienkowski (1867), Duboscq (1921), and Valkanov (1929). The following note is to be regarded as a commentary on previous work and a record of my own observations rather than as an exhaustive account of the organism.

Labyrinthula exists, as is well known, in the form of a plasmodium in which small (about 10μ long) more or less spindle-shaped units glide about in a communal ectoplasm. Each spindle has a nucleus at about its centre, and is filled peripherally with oil droplets* which may be colourless or, in some of the specimens I have studied, yellow,† imparting a yellow tinge to the whole plasmodium as seen by the naked eye (cf. Cienkowski's Taf. XV, *loc. cit.*). The number and size of the oil droplets vary considerably—and sometimes spindles are seen in which there are none at all, the cytoplasm appearing quite homogeneous. I have never observed solid food bodies in the spindles, nor contractile vacuoles. The ectoplasm surrounds each spindle with a hyaline layer which is only clearly seen when it is heaped up at some point. Here and there will be a collection of spindles visible to the naked eye as an opaque patch which may be half

* Orange after 10–15 mins. in an alcoholic solution of Sudan III, brown in 2% osmic acid. It is possibly due to the oil droplets that fixation of the spindles is so often not successful. They appear to run together, causing distortion of the cytoplasm. The best results have been obtained with neutral formalin, followed by alcohol; sometimes Bouin's fluid has given good results. Pretty preparations of spindles with the oil drops in situ are obtained by fixing in osmic acid or Flemming (with no acetic acid), staining with picrocarmine, and mounting in glycerine jelly.

† Spindles containing yellow oil may occur in the same colonies as spindles containing colourless oil.

a millimetre or more across. From these there stream out spindles, singly or in small groups, in all directions, along ectoplasmic tracks which branch and anastomose very freely. These tracks in a well established active plasmodium form a widely extended network in which the spindles circulate slowly and intermittently. It is rather rare actually to see a spindle move, but with patience and some luck it is quite possible. On one

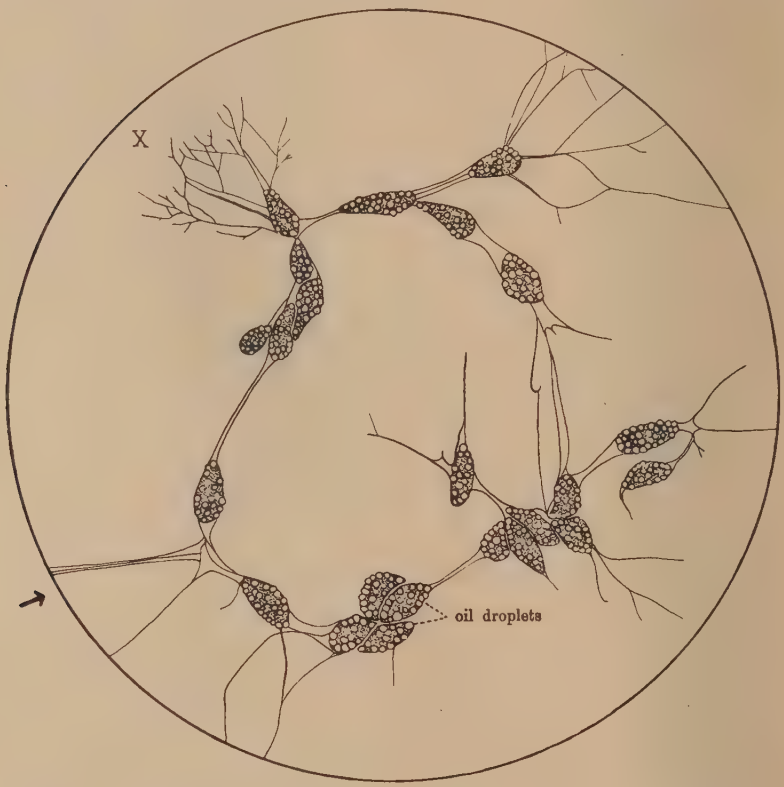

Fig. 1.—Labyrinthula spreading over coverslip in direction of arrow. At X more finely branched pseudopodia (from another specimen): these may extend all round the advancing plasmodium like an edging of fine lace. (From life.)

occasion I watched four spindles pass in succession over a length of 17μ. The times were respectively 1·5, 1·0, 2·25, and 1·25 minutes. The rate of movement is extremely variable however.

If a small portion of a deposit containing living plasmodia of Labyrinthula be removed from an aquarium on to a slide, preferably with a drop of fresh Nitzschia culture, and covered with a coverslip, the plasmodia will

creep out on to the coverslip or on to the slide, and the mode of progression can easily be studied (Fig. 1). The advancing spindles are always preceded by ectoplasmic pseudopodia, and glide along these, looking under the microscope like diminutive trams moving along their rails. There has been some discussion as to the nature of the network in which the spindles circulate (see Valkanov, 1929). Its pseudopodial nature is obvious at the edge of a spreading plasmodium ;* is there any reason for considering the more centrally lying parts as essentially different ?

Fig. 2.—Degenerating pseudopodium.

The exact relation between the ectoplasm and the spindles is obscure. The whole ectoplasm seems to be absolutely common to all the spindles, which are seen to pass one another along the tracks.

Labyrinthula feeds on diatoms and other vegetable cells. An infection

Fig. 3.—Labyrinthula feeding on Nitzschia, N. (From a living specimen.)

is in the end fatal to diatom cultures. One gets the impression that the tips of the pseudopodia gain entrance (? by natural apertures†) to the

* When degeneration sets in, as for example when the organism is observed for a long time, slowly suffocating in a preparation under a coverslip, in the light and warmth of a lamp, the peripheral parts of the reticulum may be seen to behave like other hyaline ectoplasmic structures, running into small droplets as shown in Fig. 2.

† It is well known that shelled organisms may not form their normal tests when grown in artificial culture, and it is possible that in such circumstances diatoms may offer less than the normal resistance to invasion.

cell, which is then completely used up, and the products of digestion passed back into the endoplasm of the spindles. Only very occasionally are isolated granules or droplets to be seen in the ectoplasm. When feeding

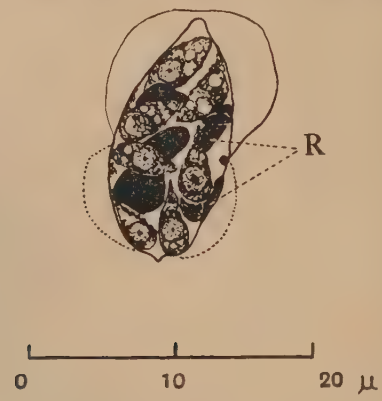

Fig. 4.—Labyrinthula in Amphiprora. From a stained specimen. R, remains of diatom contents.

on Nitzschia the spindles do not enter the diatom (see Fig. 3); but I have observed them inside a larger diatom, a species of *Amphiprora* (Ehr.) Cleve. (Peragallo, 1897–1908) as is shown in Figure 4. I was led by the

Fig. 5.—Living Labyrinthula in cells of *Laminaria saccharina*. Notice constricted spindle at X. Ectoplasm for the most part not shown.

papers of Professor O. Duboscq (1921) to infect some pieces of Laminaria with Labyrinthula. Pieces of the oarweed were placed in aquaria containing Labyrinthula, and I observed later the living plasmodia moving

through the cells of the alga (see Fig. 5) and presumably feeding on it. In my experiments the Labyrinthula seemed to obtain entrance by a damaged part of the thallus surface. It is not clear that it would otherwise have succeeded in getting in ; especially as it was closely followed by ordinarily free-living amœbæ and by ciliates. The constricted spindle at X in Figure 5 suggests, however, an ability to bore a hole in the cell

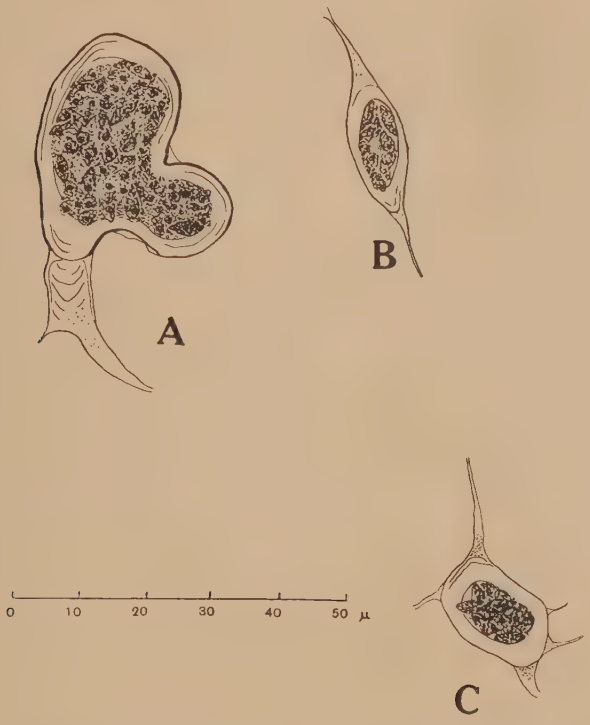

FIG. 6.—Cysts of Labyrinthula on a coverslip, from a stained preparation. Figs. B and C show two cysts evidently formed in the same plasmodium, in their original relative positions.

wall—cf. Duboscq's Figure 10. I may mention here that, though I have seen in my aquaria forms resembling other stages in the life-history of *Labyrinthomyxa* as described by Duboscq, I have not succeeded in connecting them with the Labyrinthula plasmodium. Nevertheless the two organisms appear so closely similar in their plasmodial stage that I venture to suggest that they belong to the same genus, i.e. Cienkowski's *Labyrinthula*.

The spindles multiply by binary fission, first of the nucleus and then of

the cytoplasm. The nuclear division is mitotic, with the formation of a typical division spindle.

Rounded up plasmodia are encountered, each enclosed in a thick hyaline covering of which the exact limits are difficult to see ; nor will it colour with the usual stains.* The separate spindles can be squeezed out by pressure, as indeed they can from an active plasmodium, and they have no special individual protective coats (cf. Cienkowski, 1867). Figure 6, A–C, shows in a permanent preparation what I take to be plasmodia in a similar condition, possibly some little time after their formation. Here the outer covering is brown (in Heidenhain's iron hæmatoxylin) and appears laminated. These structures are very like the cysts described by Valkanov (1929).

It is impossible to decide on a specific name for this organism. Three marine " species " of *Labyrinthula* have been made (Cienkowski, 1867 ; Valkanov, 1929), but not fully described, and it seems that all the forms may in fact belong to the same somewhat variable species. In my cultures the size of the spindles has varied considerably for example. Of the three " species " my organism fits best the description of *L. zopfi* (Valkanov, 1929), but I do not feel convinced that this is its correct name. I do not place it with *L. vitellina* Cienk. as I gather that the colour is not the same ; nor with *L. macrocystis* Cienk. as I have not observed the spore coats described by the author.

It is my pleasure once more to acknowledge my indebtedness to the late Mr. P. Jameson for cutting sections of my Labyrinthula material ; and to Mr. R. Elmhirst for help in supplying me with material both at Millport and at Glasgow.

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* No cellulose reaction was obtained in this covering with iodine and sulphuric acid ; an observation of some interest on account of the similar organisms which have been described as Mycetozoa (Zopf, 1887). It resembles the ordinary ectoplasm in its small staining capacity.

Some Additions to the Sponge Fauna of Plymouth.

By

H. J. H. Borley.

With 2 Figures in the Text.

In a small collection of sponges obtained during the winter of 1930–31 at Plymouth, six specimens were found which are of special interest. Four of these have proved to be new to the locality, the fifth was new to British waters, and the sixth was found to be a new species. Those species new to the Plymouth area are *Gellius angulatus* (Bowerbank), *Pachymatisma johnstoni* Bowerbank, *Eurypon clavatum* (Bowerbank), and *Halichondria firmus* Bowerbank. The latter is the first example of the species to be recorded since it was first described forty years ago. The species new to the British waters is *Reniera aquæductus* Schmidt, and the new species *Pseudaxinella alleni* n.sp.

Unfortunately the interest of the specimens was not fully realised at the time of collection so that only fragments of them were preserved, with the exception of *Eurypon clavatum* which was a thin plate of about 4 square mm. and of which nothing considerable remained after spicule and skeleton preparations had been made.

I must express my deep gratitude to Dr. Allen for his hospitality and very great help to me at Plymouth, and to Mr. Burton, for his invaluable assistance and advice in the writing of the following notes.

LIST OF SPECIES.

Pachymatisma johnstoni Bowerbank.

Occurrence.—Mewstone Ledge, Plymouth.

Reniera aquæductus Schmidt.

R. aquæductus Schmidt, Die Spongien des Adriatischen Meeres, 1862, p. 73, Pl. VII, Fig. 6.

Occurrence.—Mewstone Ledge.

Remarks.—The sponge is made up of 5 upright tubes, 9 cm. high by 1 cm. in diameter, fused together except at the summits. The oscules, 5 mm. in diameter, are apical and are surrounded by a peculiar raised

margin, now somewhat ragged, but probably smooth originally. The cloaca does not run through the whole tube. The surface is rather uneven in places, and minutely hispid, while the porose appearance, due to the numerous subdermal cavities, is marked. The colour in life is grey, strongly tinged with violet, and turns white in spirit. The texture is firm but compressible. The skeleton is subisodictyal, with a triangular mesh and occasional ill-defined trispiculous fibres. These fibres are seldom long and run irregularly towards the surface. The spicules are oxea measuring ·132 by ·006 mm.

The arrangement of the skeleton and dimensions of the spicules agree closely with those of a specimen of *R. aqueductus* from the Adriatic, identified by Schmidt, and now in the British Museum collection. This specimen is dried, while the present specimen is preserved in alcohol, and in this condition certain dissimilarities were observed. When a fragment of the Plymouth sponge was dried, however, the agreement between the two was found to be very close, and in this way the difference was shown to be apparent only. The Adriatic specimen is tubular, about 30 cm. high by 1 cm. in greatest diameter, narrowing slightly towards the summit and markedly towards the base. The spicules measure ·132 by ·006 mm.

The specimen originally described and figured by Schmidt, now unavailable, was an erect tube $3\frac{1}{2}$ cm. by 1 cm., and, in life, violet-blue in colour. Unfortunately, very little is known of this, and our knowledge of the species must depend mainly on the specimens from the same locality, identified by that author.

The main points of difference between the Plymouth sponge and the British Museum specimen are the more massive base and the oscular margin of the former. The presence of the oscular margin is the only considerable difference from the type. Such small differences are, however, hardly sufficient to justify a specific distinction.

Judging by Schmidt's original description the type differs also in the size of the spicules, being ·165 by ·007 mm., but here such a difference has little taxonomic value, especially as the British Museum specimen has spicules identical in size with those of the present specimen. These differences may therefore be regarded simply as evidence of the usual variation in spicule size within the species.

It is probable, judging by several specimens, including some hitherto undescribed, that the arrangement of spicules in the skeleton also shows some variation. In two examples, also in the British Museum, the one from Norway and the other from Cima Island, Madeira, both almost identical with Schmidt's type in external form, and with oxea measuring ·14 by ·004 mm. and ·149 by ·006 mm. respectively, there is a much more regular isodictyal skeleton than in the specimen identified by Schmidt referred to above.

In spite of certain minor differences, which are perhaps indicative of an ecological variation, the Plymouth sponge must undoubtedly be regarded as a representative of *Reniera aquæductus*, in which case the geographical range of the species will now include the British Isles, in addition to the Mediterranean, Madeira, Norway, and (possibly ?) Australia.

Distribution. Adriatic ; Black Sea ; Madeira ; Norway ; British Isles.

GELLIUS ANGULATUS (Bowerbank) Ridley and Dendy.

Halichondria angulata Bowerbank, Mon. Brit. Spong., 1866, II, p. 233 ; *Desmacodes angulatus* Vosmaer, Notes Leyden Mus., 1880, II, p. 107 ; *Gellius angulatus* Ridley and Dendy, Challenger Report Monaxonida, 1887, XX, p. 44 ; Lundbeck Ingolf-Exped. Rep., 1902, VI, 1, p. 62.

Occurrence.—Millbay Channel, Plymouth Sound.

Remarks.—The sponge is massive, globular and sessile, being about 5 cm. high by 10 cm. in diameter, with two oscules 5 mm. in diameter on the upper surface. The surface is very uneven and slightly hispid in places. The dermal membrane is not separable, but is supported by a confusedly subisodictyal skeleton of triangular mesh. The colour, both alive and preserved in spirit, is grey. The texture is friable. Both dermal and choanosomal skeletons are much confused subisodictyal networks with triangular mesh. The spicules are :—oxea ·199 by ·008 mm. ; toxa, ·063 by ·001 mm., with a rather rounded angle ; and minute sigmata, ·009 mm. chord.

The specimen agrees closely in form, colour and texture with the type-specimen in the British Museum. The type differs in having a more open and less confused skeleton, but the only considerable differences are a smoother surface and different spicule measurements. These are as follows :—oxea, ·225 to ·227 mm. by ·008 to ·009 mm. ; toxa, ·063 to ·072 mm. ; and sigmata, ·013 mm.

Although the general surface of the Plymouth sponge is more uneven it does in places approach the condition found in the type. Possibly the complete sponge would have shown different surface characters, but in any case this difference alone is hardly enough to justify a separation of the present specimen from *G. angulatus*.

The differences in the sizes of the spicules are also unimportant in view of the known variation within this species. The oxea vary, according to measurements given by various authors, from ·117 to ·388 mm. ; the toxa, from ·022 to ·095 mm. ; and the sigmata, from 007 to ·021 mm. The extent of the variation of the sigmata would be increased still further by the inclusion of the second set of sigmata, measuring ·078 mm., found by Lundbeck (l.c.), but the fact that this second category is only recorded

for this specimen suggests that it is an abnormal form and must be ignored for the present.

The Plymouth sponge approaches most closely those specimens described by Topsent from Luc, in which the measurements of the spicules are :—oxea, ·160 to ·260 mm. ; toxa, ·023 to ·070 mm. ; and sigmata, ·007 to ·009 mm.

This is the first record of the species from the Plymouth area, though it has been recorded from other points on the British coast.

Distribution.—British Isles ; France ; Spain ; Adriatic ; Azores ; Iceland.

Eurypon clavatum (Bowerbank).

Occurrence.—Eddystone Grounds, Plymouth.

Halichondria firmus Bowerbank.

Hymeniacidon firmus Bowerbank, Mon. Brit. Spong., 1874, III, p. 186.

Halichondria ambigua Id. t.c., p. 213.

Occurrence.—Winter Shoal, Plymouth Sound.

Remarks.—The sponge consists of thick contorted branches merging into a twisted plate. The branches bear irregular crateriform prominences each with an oscule as a minute opening in the floor of the crater. The surface is markedly glabrous except for deep irregular furrows running up the prominences and converging on the oscules. The dermal membrane is separable and supported by a rather regular network of bundles of spicules with a rhomboidal mesh. The texture is firm and fleshy. The colour in life is yellow, tinged with pink, but the pink disappears in alcohol. The skeleton is typically halichondroid, consisting of oxea, ·193 to ·369 mm. by ·004 to ·009 mm., and occasional styli ·167 by ·006 mm.

This species differs markedly from the two common British species of *Halichondria*, namely *H. panicea* (Pallas) and *H. bowerbanki* Burton, both of which are frequently met with in the Plymouth area. The characteristic features are the external form, the markedly glabrous surface, and the fleshy texture.

Some specimens of *H. panicea* approach *H. firmus* in external form, though not very closely, being distinguished by the greater regularity of the branches when present, and the usually more regular crateriform oscules. *H. bowerbanki*, on the other hand, is markedly different, but neither of these two have so glabrous a surface, and the texture of both is more friable. These two characteristics serve as a ready means of distinction. The furrowing of the surface is also a well-marked characteristic of *H. firmus*. The spicules are of similar size and shape in all three species,

but tend to be stouter in *H. panicea* and *H. bowerbanki* than in the present species.

This specimen agrees very closely with Bowerbank's description of the holotype of *Halichondria* (*Hymeniacidon*) *firmus* in all essential respects, and there can be little doubt as to the identity of the two. In point of fact it approaches more closely the type of *Halichondria ambigua* (Bowerbank), but as there appears to be no considerable difference, judged by the original descriptions, between *Halichondria* (*Hymeniacidon*) *firmus* and *H. ambigua*, there can be little doubt that the two species are synonymous.

Distribution.—British seas.

GENUS **PSEUDAXINELLA** Schmidt.

Genotype.—Pseudaxinella sulcata Schmidt.

(See Schmidt Jahresber. Comm. Unters. d. Meere 1875, V, 2–3, p. 115, and Thiele Arch. Naturg., 1903, I, 3, p. 378.)

Diagnosis.—Axinellidæ with skeleton composed of long curved styli, short bent styli, and oxea ; styli arranged in radial plumose fibres with an axial concentration ; fibres connected by oxea and short styli.

PSEUDAXINELLA ALLENI n. sp. (Figs. 1 and 2).

Occurrence.—Eddystone Grounds.

Diagnosis.—Sponge stipitate, ramose ; branches showing some anastomosis and terminating in palmate anther-like portions ; surface strongly hispid and very minutely conulose ; dermal membrane aspiculous and separable ; colour, living and in spirit, golden-yellow ; texture firm axially and soft extra-axially ; skeleton of radial plumose fibres rendering surface hispid, radiating from longitudinal concentration of fibres at axis of branch ; fibres composed of long curved styli and short bent styli and connected by short styli and few oxea ; spongin plentiful in stalk, sparse in upper branches ; spicules, long styli ·81 by ·02 mm., oxea ·27 by ·008 mm., and short styli ·343 by ·003 mm.

Remarks.—The present species agrees with the genotype in habit and the arrangement of the skeleton. It also agrees in the types and shapes of the spicules present except for the thin styli which are not mentioned for *P. sulcata.* Since these styli appear in the type specimen of the other species of *Pseudaxinella*, namely *P. egregia* (Ridley), this discrepancy is not serious. It seems probably that they are merely developmental forms and cannot therefore affect the identification. It differs further from *P. sulcata* in the comparative scarcity of the oxea and in the measurements of the spicules and from *P. egregia* in a more hispid, but less conulose

surface, a less regular skeleton, as well as in the dimensions of the spicules. The comparative scarcity of oxea may not be a real distinguishing feature in view of the tendency, shown in so many of the Axinellidæ, for the proportions of oxea and styli to vary.

The spicule differences are more striking, however, the measurements

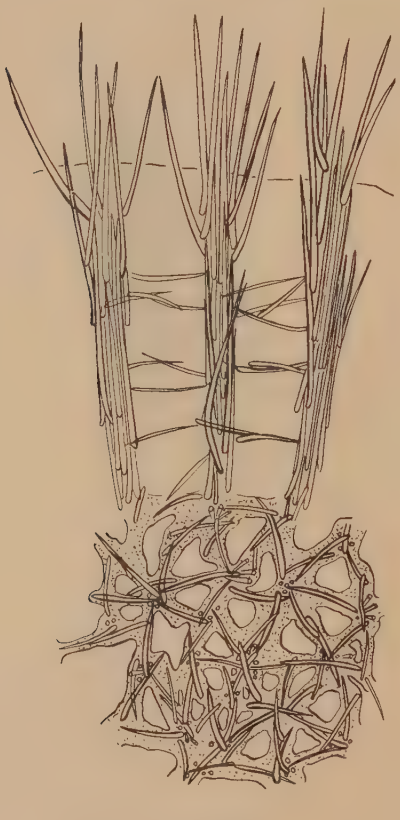

Fig. 1.—*Pseudaxinella alleni* sp. n. Section at right angles to surface.

Fig. 2.—*Pseudaxinella alleni* sp. n.; (*a*) long style; (*b*) short style; (*c*) oxeote. × 22.

for *P. sulcata* being :—long styli, 1·45 by ·02 mm.; short styli, ·45 by ·02 mm.; and oxea, ·55 to ·75 mm. by ·02 mm. In *P. egregia* the dimensions are :—long styli, ·88 to 1·207 mm. by ·012 to ·019 mm.; short styli, ·253 by ·009 mm.; and oxea, ·304 by ·012 mm.

Thus the species under consideration does not show a really close agreement with either of the two species so far recorded for the genus. Possibly

a more extensive collection of specimens might show variations in *P. sulcata* wide enough to include the new species, but in the present state of our knowledge of the genus it seems best to consider this specimen as the type of a new species.

I feel myself that I cannot do better than name this species after Dr. Allen in gratitude for his great help to me personally, and as a slight recognition of his help to other workers in the group.

Preliminary Observations on the Early Stages of *Scatella stagnalis* Fal. (Ephydridae, Diptera).

By

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With 4 Figures in the Text.

CONTENTS.

		PAGE
1.	Introduction	847
2.	Material	847
3.	Egg	848
4.	Larva	848
	(i) First Instar	848
	(ii) Second Instar	848
	(iii) Third Instar	849
5.	Pupa	850
	References	851

1. Introduction.

The insects, *Scatella stagnalis*,* for the present study were collected from the Marine Biological Laboratory at Plymouth at the kind suggestion of Dr. Allen, F.R.S., the Director of the Laboratory, during my short stay there in the summer of 1929. So far as I can see, no work on the early stages of the genus *Scatella* is available. This led me to put on record the present account of the different pre-imaginal stages of the insect *Scatella stagnalis* in the following pages. I have described here only those stages that are exhibited in my hurried collection which may not be complete. A study of its complete biology would be of considerable interest; this, however, requires a constant watch of the species at the spot for a fairly long period.

2. Material.

The insects were found attached to the growth above the water's edge on the sides of a tank in the laboratory at Plymouth. This growth consists mainly of greenish and brownish algæ which are constantly kept moist by the sea-water from the tank. The algæ, I presume, provide a suitable

* I am grateful to Dr. F. W. Edwards for the identification of the species from the adults.

feeding ground for the Scatella. Various other micro-organisms, such as the protozoa and bacteria, so common in these places, also possibly supply additional nutriment to the species.

The imagines usually lie motionless on the sides of the tanks and generally abound those tanks without rapid currents. They are small blackish flies with spotted wings. For a detailed description of the adults, reference may be made to Fallen (1823) and Berl. Ent. Zeitsch. (1895).

3. Egg.

The egg is 0·1 mm. in length and is nearly twice as long as broad. It is more or less bean-shaped, with a slight depression in the middle of the ventral side, both ends of it being somewhat rounded in the same manner and having an equal width. The micropyle is not distinguishable. The chorion is ridged with a number of transverse ripples, giving it as triated appearance, and is blackish in colour. The eggs are found singly, which suggests that they are perhaps not laid in batches.

4. Larva.

My collections show that there are at least three instars in the larval life such as are generally met with in the Cyclorrhaphous flies. I have differentiated the stages mainly on the basis of the respiratory system and it may be noted here that I have not seen their moultings.

(i) *First Instar* (Fig. 1).

The larva in this stage measures about 1·9 mm. in length with a breadth of 0·4 mm. and is whitish in colour, being more or less transparent. It consists of thirteen segments, the last segment being pushed a little ventralwards. The posterior or the anal end of the larva is truncated and is wider than the anterior end. The caudal branches of the last segment are just visible bearing a pair of spiracles at their blunt ends, one on each side ; the larva is metapneustic, there being no other spiracles. The mouth-parts agree closely with those of Ephydra (Ping, 1921). Anteriorly there is a U-shaped mandibular sclerite which is separated from the remaining parts composed of a pair of H-shaped hypostomal sclerites and a pair of lateral pharyngeal plates posteriorly.

(ii) *Second Instar.*

The second instar larva measures about 2·8 mm. in length and nearly 0·6 mm. in width. It is brownish in colour and somewhat opaque. The larva becomes amphipneustic, there having appeared a pair of thoracic spiracles now, one on each side of the prothorax, in addition to the pair

of caudal spiracles which become more pronounced than in the preceding stage. The cephalo-pharyngeal skeleton becomes highly chitinised.

(iii) *Third Instar* (Fig. 2).

The third instar or the full-grown larva measures from 5·0 mm. to 5·4 mm. in length (from tip of head to extremity of caudal branches) with

Fig. 1.—First instar larva of *Scatella stagnalis*.

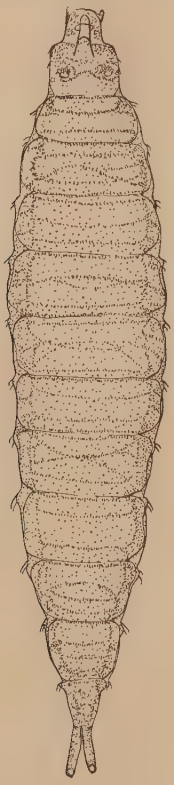

Fig. 2.—Third instar larva of *Scatella stagnalis*.

a breadth of 1·0 mm. to 1·1 mm. in the widest part; the size thus is variable to some extent. The prothoracic spiracles, one on each side, already mentioned in connection with the preceding stage become six-digited and the larva as a whole is more strongly chitinised, having an opaque brownish body surface.

It is cylindrical in shape and consists of a retractile head and twelve

body segments, possessing thirteen segments altogether. The head bears a pair of two-segmented minute antennae, the basal segment of each antenna being much wider than the distal. The body segments, excepting the two or three anteriormost, are not very distinct and are highly wrinkled. The two caudal branches in the last segment are tubular in shape, within which the main trachèæ are extended. At the apex of each of these branches lies a chitinous cap possessing the caudal spiracle in the centre and four fan-shaped membranous structures hanging laterally.

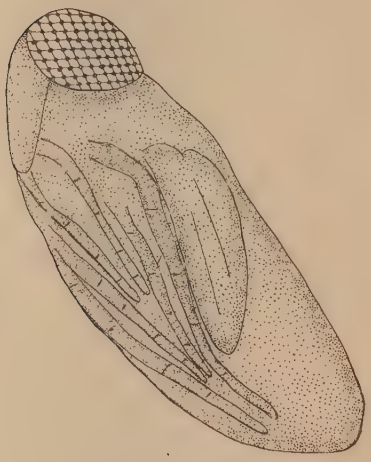

Fig. 3.—Pupa of *Scatella stagnalis*.

Fig. 4.—Puparium of *Scatella stagnalis*.

The body is thickly beset with spines which are more prominent dorsally. Each of the first eight abdominal segments bears a pair of nipple-like prolegs which are provided with a number of short setæ. The last pair of prolegs are larger than any of the preceding ones.

5. Pupa (Fig. 3).

The pupation takes place within the last larval skin which functions as the puparium (Fig. 4). The anterior three or four segments of the puparium are flattened dorso-ventrally, and posteriorly the puparium is more or less barrel-shaped. The pupæ are attached to the substratum by means of the caudal branches in the puparium and the branches at this stage diverge laterally and are not directed posteriorly as in the larva.

The pupa is much smaller in size than the puparium, being nearly half

the latter, and is enveloped in a thin transparent saccular sheath through which the imaginal appendages can be clearly distinguished. The pupa measures about 2·3 mm. in length with a width of 0·9 mm.

The anterior end of the pupa is broader than the anal end, and the head possesses a pair of prominent eyes. A pair of small antennal tubercles can be seen at the sides of the head in front of the eyes. The proboscis is flattened and firmly pressed to the body in between the coxæ of the legs. The wings and the three pairs of legs are also closely pressed against the ventral surface of the pupal body.

The imago, when ready to emerge, effects a split at the anterior flattened end of the puparium, through which it subsequently escapes. The split runs along the sides of the puparium, dividing this end of the puparium into two almost similar flaps, one dorsal and one ventral.

The species seems to be fairly free from enemies, as most of the parasitic and predaceous insects find the salinity of the water, in which the Scatella abounds, unsuitable for habitation. Certain mites, however, are often found in the beds of *Scatella stagnalis*.

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A New Type of Luminescence in Fishes, III. The Gland in *Cœlorhynchus cœlorhynchus* Risso.

By

C. F. Hickling, M.A.

With 3 Text-Figures and Plates I–IV.

In 1925 I published a paper (**1**) describing a new type of luminescence in fishes, exhibited by the Macrurid fish *Malacocephalus lævis* Lowe, and a further paper (**2**) described experiments made on the luminescent secretion of this fish. In my first paper, I remarked that the Macrurid *Cœlorhynchus cœlorhynchus* Risso also possesses, "between the pelvic fins, a naked, pigmented patch; resembling that of *M. lævis*, and dissection again reveals a flat, pigmented sac lying in the body-wall adjacent to this pigmented patch." "It gives one the impression of a rudimentary organ, yielding no secretion, but connected with the anus by a pigmented, functionless duct." The present paper describes this gland in *C. cœlorhynchus*.*

Farran (**3**) writes, of *C. cœlorhynchus*, "this species is found in the Mediterranean, and in the Atlantic for about 20° to north and south of the Straits of Gibraltar, occurring along the edge of the continental shelf, and descending for a short distance down its upper slopes." I have recorded it (**4**) from the West Coast of Scotland, and Smitt (**5**) includes it among the fishes of Norway. In my first paper, I stated that I had then only seen five specimens on the Hake grounds south-west of Ireland, but, in 1926, I was able to examine many specimens at sea, and to cut out and fix the glands for further examination ashore. Unfortunately, I did not measure these specimens, which, however, were all comparatively large, certainly not less than 30 cm. in length. They will be referred to, in this paper, as "older specimens." Microscopic examination of these glands showed much variation in structure, and I felt that a proper understanding could be attained only after a comparison with younger specimens. I had to wait until August, 1930, and January, 1931, for this material. In August, 1930, the Ministry of Agriculture and Fisheries' research vessel *George Bligh* took two specimens, 14 and 15 cm. in length, on the "West-

* The work has been done in the writer's spare time, with material collected during voyages on hake research.

ward Ground," in about 180 fathoms, latitude about 49° 50′ N., and in January, 1931, the steam trawler *Tenedos*, aboard which I was engaged in hake research, by courtesy of the owner, Mr. H. E. Rees, took many specimens between 18 and 23 cm. in length, and one of 30 cm.

Some specimens were preserved whole, others were dissected, and the ventral gland, with its associated structures, fixed in Bouin's Fluid. The fixed material was cut into sections and stained by Mr. B. G. Clarke, Chief Laboratory Assistant at the Lowestoft Laboratory, to whom I would here acknowledge my thanks for his care and skill.

The stains used were Iron Hæmotoxylin and Eosin. The material was not easy to cut, owing to the abundance of tough connective tissue, and the sections have not always been perfect.

Finally, I would thank Professor Carl L. Hubbs, of the University of Michigan, for the benefit of his expert advice concerning the Macruridæ. In using his correspondence, I wish to avoid casting any responsibility upon him for my own findings.

A. The Anatomical Relations of the Gland.

Farran (**3**) writes, of *C. cælorhynchus*, "between the ventral fins is a scaleless, oval depression, black in colour." Smitt (**5**) gives a very fine figure of this fish, and describes "a narrow, oblong, bare spot in the median line of the belly between the ventral fins." In his figure, the scaleless, oval spot, or depression, is placed with its centre decidedly anterior to a line joining the bases of the pelvic fins. This was also the usual position in my larger specimens. In my smaller specimens, the depression tended to lie more directly between the bases of the fins.

There is much bluish-black colouring in the skin in the ventral region of the fish, and the scaleless spot occurs in the midst of this, as a distinct, spindle-shaped depression. The margins of the depression project slightly, and the floor of the depression is itself covered with a comparatively thin skin, thickly strewn with melanophores. The posterior half or two-thirds of the depression bulge slightly.

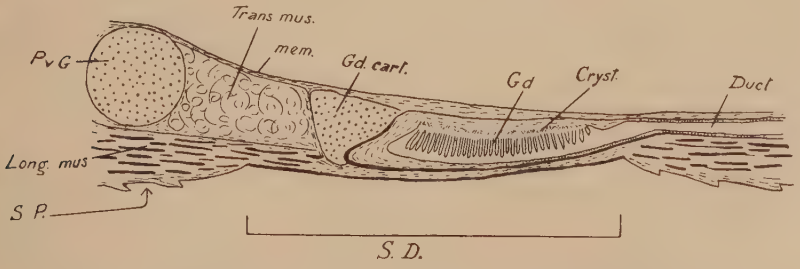
Posteriorly, the depression ends indefinitely, but a well-marked trail of pigment runs backwards from it, between the pelvic fins, to the anus, which is itself surrounded by a slightly swollen area heavily loaded with melanin.

From the dorsal aspect, the body-wall in the region anterior to the anus is seen to be covered by the thin silvery peritoneum, speckled with melanophores, and, when this is stripped off, a gland is revealed, lying in the mid ventral line, appearing as a swelling, well covered with black pigment and silvery guanin, of oval shape, tapering posteriorly into a duct likewise well wrapped in black pigment. Gland and duct are sur-

rounded by the muscles of the body-wall, and are overlaid by a very tough, almost cartilaginous membrane, of stoutly fibrous connective tissue (Text-Fig. 1 : *Mem.*). This membrane runs forward, and is connected to a prominent, curved ridge, which marks the junction of the right and left pelvic bones with the median pelvic cartilage (Text-Fig. 1 : *Pv. g.*). Through this membrane, two small blocks of cartilage may be seen associated with the gland : these cartilages meet in the middle line above the anterior extremity of the gland (Text-Fig. 1 : *Gd. cart.*).

Text-Figure 1 is a drawing of a rough sagittal section of the gland, made with a razor, and viewed through a lens.

The gland (*Gd.*) is a flattened sac overlying the scaleless depression (*S. d.*) ; it is partly filled with a brilliantly white substance, and is surrounded and defined by thick sheaths of black pigment, distinct from the



Text-Fig. 1.—Rough sagittal section of the ventral gland and the adjacent structures in a 30-cm. specimen of *C. cœlorhynchus*.
For explanation of the lettering, see text.

black pigment present in the floor of the scaleless depression. Both anterior and posterior to the scaleless depression, the scale-pockets (*S.p.*) are shown.

Posteriorly, the gland tapers away into the duct (Text-Fig. 1 : *Duct*) which runs backward to the anus. Anteriorly, the gland tapers obliquely downwards, to end bluntly, like the toe of a shoe. Text-Figure 1 was made from a specimen of 30 cm. In smaller specimens (for example, of 15 cm., Plate I, Fig. 1) the gland ends roundly, anteriorly, without tapering. This point is dealt with in Section C. In all cases, however, the connective tissue capsule of the gland is strongly bound to the peculiar cartilages (Text-Fig. 1 : *Gd. cart.* ; Plate I, Fig. 1 *et seq.*) mentioned above. Further forward, the section in Figure 1 cuts through the median cartilage of the pelvic girdle (*Pv. g.*) ; between the pelvic girdle and the gland cartilages lie transverse muscles (*Trans. mus.*), and longitudinal muscles lie beneath these (*Long. mus.*).

The gland cartilages are closely associated with the pelvic girdle. In the

first instance, they are bound, and the gland itself with them, to the pelvic girdle, by the overlying membrane of stout connective tissue (Text-Fig. 1 : *Mem.*), but, they are also more directly connected with the posterior processes of the pelvic girdle.

Text-Figure 2 is a reconstruction of the pelvic girdle in *C. cœlorhynchus*. The specimen was of 23 cm., and ossification in the girdle was incomplete.

There is a comparatively large, wedge-shaped median cartilage (*M. c.*) lying in the mid-ventral line, to which the pelvic bones of either side are attached (*P.*). From the posterior region of each pelvic bone, anterior to the point where these expand to form the articulation (*Art.*) of the fin-rays, springs a comparatively stout posterior process (*P. p.*, using the nomenclature of Goodrich, **6**) which inclines in a curve towards the middle

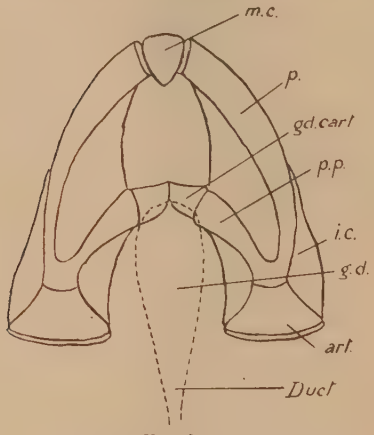


Fig. 2.

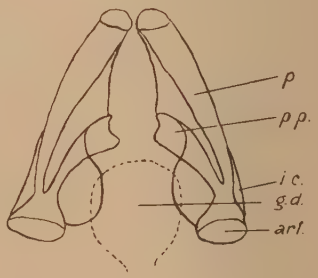


Fig. 3.

Text-Figs. 2 and 3.—Pelvic girdles of *C. cœlorhynchus* of 23 cm. (Fig. 2) and *M. lævis* of 25 cm. (Fig. 3).

line. Each posterior process is bound (but not, apparently, fused) to its corresponding gland-cartilage (*Gd. cart.*). The two gland cartilages curve upwards and towards the middle line, forming a bridge which spans the anterior of the gland. They meet at the middle line, and are bound to each other, and to the connective tissue of the gland, by a strong ligament (Plate II, Fig. 3).

A shallow crest (*I. c.*), which we may call the iliac process, runs dorsally along the pelvic bones of each side, from the angle of the articulation of the fin-rays to a point about half-way along the shaft of the pelvic bone. The posterior process is united, both to the pelvic bones and to the inner (medial) angle of the articulation, by membranes in which ossification was well advanced in the young specimens dissected.

B. The Structure of the Gland in Young Specimens.

Plate I, Figure 1, is a drawing of a sagittal section of the gland and duct in a 15-cm. specimen of *C. cœlorhynchus*. Its general features resemble those of the gland of the 30-cm. specimen in Figure 1. The gland lies in the thickness of the body-wall : above is the body-cavity. The gland is strongly bound with fibrous connective tissue (*C. t.*) which also binds it to the gland cartilage (*Gd. cart.*). In the neighbourhood of the cartilage, the fibres of the connective tissue are very thick, and stain deeply with Iron Hæmotoxylin. Transverse or oblique muscles (*Trans. mus.*) are seen anterior to the gland-cartilage. The scaleless depression can be discerned in the outline of the outer skin of the fish (the lower margin of the section). In this young specimen, the gland overlies the whole of the scaleless depression.

The sheet of stout connective tissue overlying the gland (shown in Text-Fig. 1) is not seen in this section ; it has probably become detached, with the peritoneum, during manipulation. The connective tissue in the roof of the gland is thickly impregnated with guanin crystals ; these reflect the light strongly, and make this region conspicuous. They constitute the "brilliant white substance" observed in the rough sagittal section of the gland. The walls of the gland also contain a continuous layer of melanophores : these are especially abundant in the floor of the gland (*Mel.*).

From the roof of the gland a system of tubules (*S.t.*) projects downwards ; these gradually become confluent into irregular collecting spaces (*C. s.*), which are traversed by trabeculæ of connective tissue with an epithelial lining, and are confluent posteriorly with the duct (*d.*). The tubules are actively secretory ; they are lined with a conspicuous epithelium secreting dense masses of minute granules. They seem to resemble, in all respects, those of *M. lævis* (**1**), and the appearance and formation of the secretion is also similar (**2**). A number of small blood-vessels (*B.v.*) are present in the roof of the gland, which branch into very fine vessels passing down into the walls of the tubules.

Plate II, Figures 3, 4, and 5, are drawn from a series of sections cut horizontally through the gland of a 14-cm. specimen of *C. cœlorhynchus*, i.e. parallel to the ventral surface of the fish. Figure 3 is a section just touching the extreme top of the gland, Figure 4 is a section through the upper portion of the gland, and Figure 5 a section near the base of the gland.

The greater part of the space in Figure 3 is occupied by the gland-cartilages (*Gd. cart.*) which almost touch each other at the middle line. They are separated by a few fibres of the stout ligament which binds them together, and to the connective tissue capsule of the gland (*C.t.*). The

fibres of this ligament stain deeply with Iron Hæmotoxylin. The extreme top of the gland is seen posterior to (below in the figure) the gland cartilages. It consists of connective tissue, containing many melanophores (*Mel.*), an area rich in crystals of guanin (*Cryst.*), and numerous much-branched blood vessels (*B. v.*). It is clear that this series of sections is slightly oblique, since the left gland cartilage is cut more longitudinally, the right, more transversely ; this is confirmed by Figure 4, cut at a lower level of the gland, where the left gland cartilage has passed out of the section, while the right, cut more longitudinally as it curves outwards and downwards, is still visible.

In Figure 4, as has just been stated, the right gland cartilage (*G. c*). is seen, attached to the wall of the gland, which contains its usual melanin sheath (*Mel.*) and blood vessels (*B. v.*). The gland itself consists, at this level, almost entirely of the secretory tubules (*S. t.*), here cut transversely. At the anterior end, however, the lumina of these tubules are already running together into collecting spaces (*C. s.*). Longitudinal and transverse muscles surround the gland.

In Plate II, Figure 5, the secretory tubules are no longer present, and the gland consists, at this level, of the collecting spaces (*C.s.*) which are confluent, posteriorly, in the Duct (*D.*). A very complete melanin sheath (*Mel.*) surrounds both gland and duct. There is a constriction at the junction of the gland and duct, and, at first, I suspected some kind of sphincter mechanism : no muscle is apparent, however, and it is probably a fortuitous projection of the wall of the duct.

C. The Structure of the Gland in Older Specimens.

The structure of the gland of *C. cœlorhynchus*, as it is found in small specimens of 14 and 15 cm., may now be compared with that of the gland in larger specimens, whose length, not ascertained, may almost certainly be put at over 30 cm.

In Plate I, Figure 2, is a drawing of a sagittal section through the gland of an older specimen. It is drawn to the same scale as Plate I, Figure 1.

The structure of the gland resembles that of the young fish. The gland cartilage is present, but is out of the field to the right. The gland consists of a connective tissue capsule, bearing melanophores (*Mel.*), and containing secretory tubules (*S. t.*) discharging into collecting spaces (*C. s.*). The tubules are full of secretion.

The striking difference between the larger and the smaller specimens lies in the fact that the gland, in the larger specimen, is about twice the length and depth of that of the smaller, whereas the secreting portion occupies about the same area in both fish. In the larger fish, the connective tissue of the roof has increased greatly in thickness, and the layer

of guanin crystals (*Cryst.*) is separated from the tubular portion of the gland, upon which it abuts in the smaller specimen. The system of collecting spaces occupies a much greater area in the larger fish, and runs far forward, beyond the secretory tubules, as a blind pocket. The melanin sheath is less conspicuous, the pigment is distributed more thinly, almost as if the increase in size of the gland had not been accompanied by an increase in pigmentation.

In Plate III, Figures 6 and 7, are shown, in transverse section, the glands of two older specimens of *C. cœlorhynchus*.

The gland in Figure 6 is, clearly, very similar to that shown in Plate I, Figure 2; there is a well-developed system of secretory tubules (*S. t.*) containing abundant secretion, a well-marked melanophore sheath (*Mel.*), and large collecting spaces (*C. s.*). The tubular portion of the gland, however, is reduced in area as compared with the gland of a smaller fish such as that in Plate I, Figure 1, and appears, in fact, to occupy an even smaller space than that in the gland shown in Plate I, Figure 2, while there is a tendency for the secretory tubules to project obliquely, rather than vertically, towards the collecting spaces.

In Figure 7, the gland has undergone great reduction. The secretory tubules have disappeared, the gland is very much flattened, and contains only collecting spaces (*C. s.*), themselves reduced and flattened. The melanophore sheath is very indistinct (*Mel.*). One of the gland-cartilages (*Gd. cart.*) is visible, bound to the gland by deeply staining fibrous connective tissue.

Three other glands of older specimens were sectioned and examined. In all three there was a relative reduction in the secretory portion of the gland, as compared with a small specimen, and the blind forward prolongation of the collecting spaces, shown in the section in Plate I, Figure 2, is a common feature.

Comparing the structure of the gland in smaller and larger specimens, it appears that, with an increase in the size of the gland, there is no corresponding increase in the amount of the secretory epithelium, and a greater diffusion of the pigmentation of the gland. This points, in my opinion, to disuse and degeneration of the gland as the fish grows larger.

D. The Duct.

It has already been mentioned that the gland narrows posteriorly into a duct, which runs backward to the anus, and is marked by a trail of black pigment. The duct may be seen in Text-Figure 1, in Plate I, Figures 1 and 2, and in Plate II, Figure 5. It is lined with epithelium two or three cells deep, and is, in smaller specimens at least, surrounded with melanophores.

Its length varies with the size of the fish, not only absolutely, as may be expected, but relatively also.

This may be seen in the measurements given below.

Length of Fish.	*Length of Duct.*	$\frac{\textit{Length of Fish.}}{\textit{Length of Duct.}}$
15 cm.	0·5 cm.	30
18 cm.	0·9 cm.	20
19 cm.	0·8 cm.	24
20 cm.	0·8 cm.	25
22 cm.	0·9 cm.	24
22 cm.	0·9 cm.	24
30 cm.	2·0 cm.	15

In the fish of 15 cm., the length of the duct is only one-thirtieth of that of the fish, in one of 20 cm., one-twenty-fifth, and in one of 30 cm., one-fifteenth. This relative lengthening of the duct seems to be associated with a relative forward movement of the pelvic fins, accompanying the growth of the fish.

The duct runs backward to the anus. Its final course there may be traced by examining a series of horizontal longitudinal sections, cut transversely to the rectum, and parallel to the surface of the body in the neighbourhood of the anus.

Drawings are shown, in Plate IV, of sections from such a series in specimens of 21 cm. (Fig. 8), and of 30 cm. (Fig. 9). Both are drawn to the same scale.

In Figure 8, the rectum (*R.*) occupies the centre of the section, lined with its characteristic tall and irregular epithelium. It is surrounded by connective tissue, containing blood vessels (*B. v.*) and melanophores (*Mel.*). The sphincter muscle is not seen in this section. Surrounding the rectum is a system of loose spaces (*P.*) lined with an epithelium closely resembling that of the duct of the ventral gland, but quite distinct from that of the rectum. Surrounding these spaces is a second, and denser ring, of melanophores. Posterior to the anus, there appears, cut in transverse section, the genital duct (*G. d.*), here appearing as a space in the connective tissue, and the kidney duct (*K. d.*) with a well-defined columnar epithelium.

In Figure 9, which is a section cut at a somewhat higher level, in its series, than that shown in Figure 8, the rectum again occupies the centre of the field (*R.*), and the ring of unstriped muscle (*Mus.*) is conspicuous in its wall. Outside this is a dense ring of melanophores (*Mel.*), which surrounds the rectum, and also surrounds a system of spaces (*P.*) lying in the connective tissue surrounding the rectum. Anteriorly (to the right in the figure) the ring of melanophores narrows to a sheath surrounding the

duct of the ventral gland; the latter is shown, cut obliquely, at (*D.*). Posteriorly, the genital duct (*G. d.*) and kidney duct (*K. d.*) are to be seen.

These series of sections show that the duct of the ventral gland widens, at the rectum, into a series of loose spaces, which more or less surround the rectum, and end blindly, dorsally, in irregular pouches. The pouches extend relatively much further up the sides of the rectum in the smaller specimen (21 cm.) than in the larger specimen (30 cm.).

These pouches, with their melanin sheath, are the cause of the "slightly swollen area, heavily pigmented," which may be seen, with the naked eye, surrounding the anus.

E. A Comparison with the Gland and Duct in *M. lævis*.

There can be no doubt but that the ventral glands in *C. cœlorhynchus* and *M. lævis* are homologous. In both, the duct opens to the exterior "about the anus, in such a way as to surround the lower part of the rectum" (1). In both, pouches arise from this external opening, which run upwards beside the rectum, and end blindly. In both cases, the secretory part of the gland consists of tubules, which discharge a granular secretion into a system of loose collecting spaces. As pointed out in *M. lævis*, these tubules are best regarded as "an epithelium, which has been thrown into long folds." The tubules and collecting spaces are firmly bound in connective tissue, to form a compact gland, containing a layer of guanin crystals, and insulated from the other tissues of the fish by a screen of melanophores. The gland lies, in both cases, in the thickness of the body-wall, forward of the rectum, and between the pelvic fins.

There are some differences. Firstly, the duct is very short in *M. lævis*, relatively long in *C. cœlorhynchus;* but there is reason to think that this difference becomes less significant in small specimens (Section D). The gland in *C. cœlorhynchus* is notably flatter in shape than that of *M. lævis;* the secretory tubules of *C. cœlorhynchus* project downwards from the roof of the gland, in *M. lævis* they project towards the centre of the gland, mainly from the anterior and lower walls. The gland of *M. lævis* overlies two small scaleless depressions in the skin: one large depression is associated with the gland in *C. cœlorhynchus*. From their respective relations with the gland, the two small depressions in *M. lævis* are probably homologous with the single large depression of *C. cœlorhynchus*. The significance of these depressions might possibly be explained by an embryological study, for which the materials are at present lacking.

These differences are trifling, and might be much less marked in small fish. There are three important differences.

Firstly, the gland in *M. lævis* is furnished with unstriped muscle, which forms a sheet over the top of the gland, "probably closely connected

with the function of compressing the gland to cause emission of the secretion." There is no such muscle even in young specimens of *C. cœlorhynchus*.

Secondly, the gland in *C. cœlorhynchus* is strongly bound, at its anterior end, to two special cartilages, which are connected, in their turn, with the pelvic girdle (Text-Fig. 2). These are present in older specimens of *M. lævis*, but the gland is not closely bound to them, as it is in *C. cœlorhynchus*. The pelvic girdle of a young (25 cm.) specimen of *M. lævis* is shown in Text-Fig. 3. Comparing it with the pelvic girdle of a 23 cm. specimen of *C. cœlorhynchus* it is clear that there are considerable differences. The median cartilage is wanting in *M. lævis*, and the posterior processes, which in *C. cœlorhynchus* are comparatively stout rods, in *M. lævis* are flattened, less definite structures, from which arise sheets of bone, which, arching upwards (dorsally) and towards the middle line, form a shallow cavity in which the gland (of which the outlines are indicated) is contained. In an older specimen of *M. lævis* (43 cm.) the posterior processes are more rod-like, as in *C. cœlorhynchus*, and two very small rounded cartilages are present, which connect the two posterior processes of the pelvic girdle across the middle line. But the luminiferous gland appears to be quite independent of these cartilages.

It seems probable that the "lens-like body" figured and described (1) as lying anterior to the gland, in *M. lævis*, is really hyaline connective tissue filling the space between the gland and the enclosing sheets of bone, and in no way "part of an optical apparatus, for casting light from the gland itself," as I doubtfully suggested in my paper on *M. lævis*.

These differences between the relations of the gland and the pelvic girdle are probably associated with a different mode of ejection of the secretion. If ejection is brought about, in *M. lævis*, by the action of the unstriped muscle in the wall of the gland, the sheets of bone, enclosing the anterior portion of the gland, will give the necessary support against which the pressure produced in the gland by the muscle can have effect. In *C. cœlorhynchus*, it seems probable that ejection is accomplished on quite a different plan. The two gland cartilages rise up in an arch, spanning the fore part of the gland, and are united above, at the middle line, by a ligament. This ligament might act as a hinge, so that, if the lower ends of the gland cartilages were pressed towards each other by the posterior processes of the pelvic girdle, to which they are attached, the gland would be squeezed, nutcracker-fashion, and the contained secretion forcibly ejected. Thus ejection could be brought about by the transverse muscles between the two halves of the pelvic girdle, acting on the gland by way of the posterior processes and the gland cartilages.

The third difference is this, that the gland of *M. lævis* remains actively functional, in producing a luminescent secretion, throughout life, whereas,

in *C. cœlorhynchus*, the evidence points to its degeneration with growth. I have not found, among scores of specimens of all sizes, a single *M. lævis* which did not yield abundant brilliantly luminescent secretion on the gentlest pressure applied to the pelvic region. The glands of larger specimens of *C. cœlorhynchus* yield no luminescent secretion, though the gland, newly cut open, may show a very feeble luminescence. In a darkened cabin, I held in one hand a small (20 cm.) specimen of *C. cœlorhynchus*, in the other a 25-cm. specimen of *M. lævis*, and squeezed their ventral glands simultaneously. *M. lævis* instantly yielded a large drop of brilliantly luminescent secretion: *C. cœlorhynchus*, after more vigorous treatment, showed a thin smear, barely visible to the dark-adapted eye.

I have no doubt that the gland on *C. cœlorhynchus* is fully functional in the very small fish, and is probably used in the same way as that of *M. lævis*. But I also have no doubt that, in older specimens, the gland becomes more or less vestigial.

The gland of *M. lævis* is more primitive, or less specialised, than that of *C. cœlorhynchus*, especially in the connection of the gland, in the latter, with the pelvic girdle. If the gland is indeed derived from "a glandular area about the anus, which has become invaginated to form a pouch, and secondarily folded to give an enormous internal area of secretion," the ejecting mechanism in *M. lævis*, consisting of unstriped muscle such as could have been carried in from the rectum, is decidedly more primitive than the association with the pelvic girdle, which we find in *C. cœlorhynchus*, and which must be regarded as a secondary development.

In the adult *Trachyrhynchus trachyrhynchus* Risso, there is no sign of a ventral gland, even in sections of the rectum and its surrounding tissues, and Johnsen (**7**) makes no mention of its presence in young bathypelagic specimens. Had a ventral gland been present, Johnsen could not have failed to mention or investigate it.

F. The Ventral Gland in the Anacanthini.

In my paper on *M. lævis*, I suggested that the gland in *C. cœlorhynchus* was degenerate, and that this might be associated with a stouter armature of scales in the latter species, "which has rendered unnecessary the protective device of a luminous organ." I also suggested that "the organ may be widely spread among Macruridæ, and further work on other species might reveal its presence." The suggestion was ill-informed. The ventral gland is widely spread among the Macruridæ. Gilbert and Hubbs (**8** and **10**) describe its presence in many genera, such as Cœlorhynchus, Abyssicola, Hymenocephalus, Malacocephalus, Ventrifossa, etc. They used it in their classification of these fishes, and suggested that it

might be a phosphorescent organ. Still earlier, Radcliffe (**9**) had suggested that the ventral gland found in *Macrurus lucifer* (= *Ventrifossa lucifer*, Smith and Radcliffe) was phosphorescent.

The macroscopic structure of the ventral gland in the Macruridæ, as described by Gilbert and Hubbs (**8** and **10**), shows great variation. The gland may be sing'e (as I have found it in *M. lævis* and *C. cœlorhynchus*), or double, a second gland being present at the anus. Gilbert and Hubbs (**10**) thus describe the gland in *Cœlorhynchus argentatus*. "The posterior dilatation is bilobed, being divided by the anus. The thick anterior dilatation is roughly triangular in outline . . . it lies within a cavity, and is supported in a strikingly peculiar manner by a cartilaginous rod in close connection with the pelvic girdle. The posterior arm of this bone is a poorly ossified plate, which, by meeting its fellow at the median line, forms a brace directly between the pelvic bases. From the anteromedian angle of each of these posterior limbs, a cylindrical rod of cartilaginous tissue extends forward to the sides of the anterior dilatation, from which it extends dorsad, meeting its fellow in a wide arch, the apex of which is bound to the well-ossified anterior arms of the public (*sic*) bone, where these meet at the middle line." The structure of the gland and cartilages, in their relation to the pelvic girdle, in *C. argentatus* obviously very closely resemble those of *C. cœlorhynchus*. The posterior gland, divided by the anus, described in the former, and in many other species, is plainly to be homologised with the pouches which arise from the duct, about the rectum, in *M. lævis*, and *C. cœlorhynchus*. Hubbs himself, in a letter to me, admits of no doubt that the gland is homologous throughout the group of Macrurids.

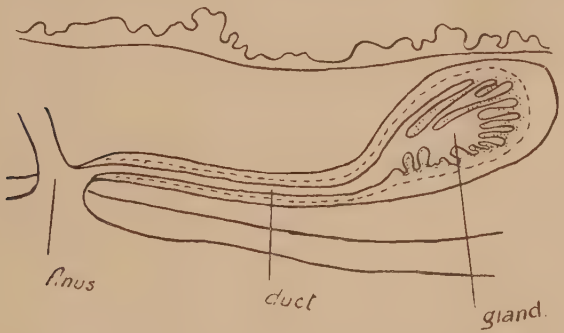
A closely similar gland is present in a genus of the Gadidæ, namely, Physiculus. Franz (**11**) has described briefly the ventral gland in *P. japonicus*. I take the liberty of reproducing his figure below (Text-Fig. 4).

A round, black, scaleless fossa lies in the median line before the anus. "Hierunter eine schöne Drüse im Muskelfleisch eingebettet liegt. Langschnitte . . . zeigten mir ferner, dass der ausfuhrungsgang der Drüse in den After mündet. Die Drüse . . . besteht aus gestrickten, radiär gestellten Drusenschlaüchen, und nicht etwa . . . aus einen gefalteten Drüsenepithel. . . . Das Epithel und überhaupt die Schleimhaut des Ausfuhrungsganges zeigt . . . starke längsfalten." Jordan and Hubbs (**12**) found a similar fossa in three American species of Physiculus, namely, *P. fulvus*, *nematopus*, and *rastelliger*, which makes it probable that a ventral gland is present in these species also.

There can be no doubt that this gland is homologous with that of the Macruridæ: it is present, therefore, in both families of the Anacanthini. As far as I know, it has not been found in any other Gadoids.

Boulenger (**13**) and Regan (**14**) are both of the opinion that the Macruridæ are more primitive than the Gadidæ. I would suggest that the gland arose in the primitive Anacanthini, probably during an invasion of deep water, that it has been retained and elaborated in the Macruridæ, which have persisted in the deeper water, where the gland is presumably of use, but lost in the Gadidæ, with the exception of the genus Physiculus.

Owing to the variation in the structure of the gland among living



Text-Fig. 4.—Sagittal section of the ventral gland in *Physiculus japonicus*, after Franz.

Macruridæ, and the fact that it appears to degenerate in some species, it should probably be regarded as essentially a larval or post-larval organ, which may remain functional throughout life in some species. Smitt (**5**) described what is clearly the fossa underlying the ventral gland, in a post-larval Macrurid, but suggested that it might be an adhesive organ.

A comparative study of the structure of the gland in a wide range of Macrurid and Gadoid fishes might throw some light on their mutual relationships.

SUMMARY.

The luminiferous organ of the Macrurid fish *Cœlorhynchus cœlorhynchus* Risso is described in this paper. It consists of a gland, flattened dorso-ventrally, placed in the body-wall, just in front of the pelvic fins. The secretory epithelium is thrown into a series of tubules, projecting downwards from the roof of the gland into a system of collecting spaces : the latter are confluent, posteriorly, with a duct which leads backwards, between the pelvic fins, to the anus. Blind pouches, which run upwards for a short distance beside the rectum, arise from the duct at its external opening at the anus.

The gland itself, which is compactly bound in fibrous connective tissue rich in melanophores, is strongly bound to a pair of cartilages, which rise dorsally to span the anterior end of the gland, are united by a ligament at the middle line, and are connected, lateroventrally, to the posterior processes of the pelvic girdle. It is suggested that contraction of the muscles between the two halves of the pelvic girdle might cause the gland cartilages to squeeze, nutcracker-fashion, the anterior part of the gland, the ligament binding the cartilages together at the middle line acting as a hinge. It is probable that the fish actively expels the luminiferous secretion of the gland by this means.

The gland is apparently functional only in the young fish : it becomes more or less vestigial in larger specimens.

The structure of the gland in *C. cœlorhynchus* is compared with that of the homologous gland in the Macrurid fish *Malacocephalus lævis* Lowe ; the gland of the latter is the more primitive or the less specialised.

Finally, the glands of these fishes are homologised with those in other Macruridæ, and in the Gadoid genus Physiculus. It is suggested that a comparative study of the structure and distribution of these glands might be of help in establishing the relationships of the members of the Anacanthini.

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EXPLANATION OF THE LETTERING USED IN PLATES I–IV.

B. v., Blood vessels. C. s., Collecting space. Cryst., Guanin Crystals. C. t., Connective tissue. D., Duct of gland. Gd. cart., Gland cartilage. G. d., Genital duct. K. d., Kidney duct. L. mus., Longitudinal muscle. Mus., Muscle. Mel., Melanophores. P., Pouches arising from duct of gland. R., Rectum. S. t., Secretory tubules. Trans. Mus., Transverse muscles. The scales accompanying the figures are one millimetre, divided into tenths.

PLATE I.—Sagittal sections through the gland and duct of *C. cœlorhynchus*.

FIG. 1.—In a 15-cm. specimen.

FIG. 2.—In an older specimen.

PLATE I.

PLATE II.—Horizontal longitudinal sections through the gland of a 14-cm. specimen of *C. cœlorhynchus*.

FIG. 3.—Through the top of the roof of the gland.

FIG. 4.—Through the tubular portion of the gland.

FIG. 5.—Through the floor of the gland.

trans mus.
Ligament.
Gd. cart
mel
c.t
cryst
mel
b.v
3
c.s
mel
L mus
b.v.
d
5.
trans mus
c.s
Gd. cart
s.f
L mus
mel
b.v
4

PLATE III. FIGS. 6 AND 7.—Transverse sections through the gland in two older specimens of *C. cœlorhynchus*.

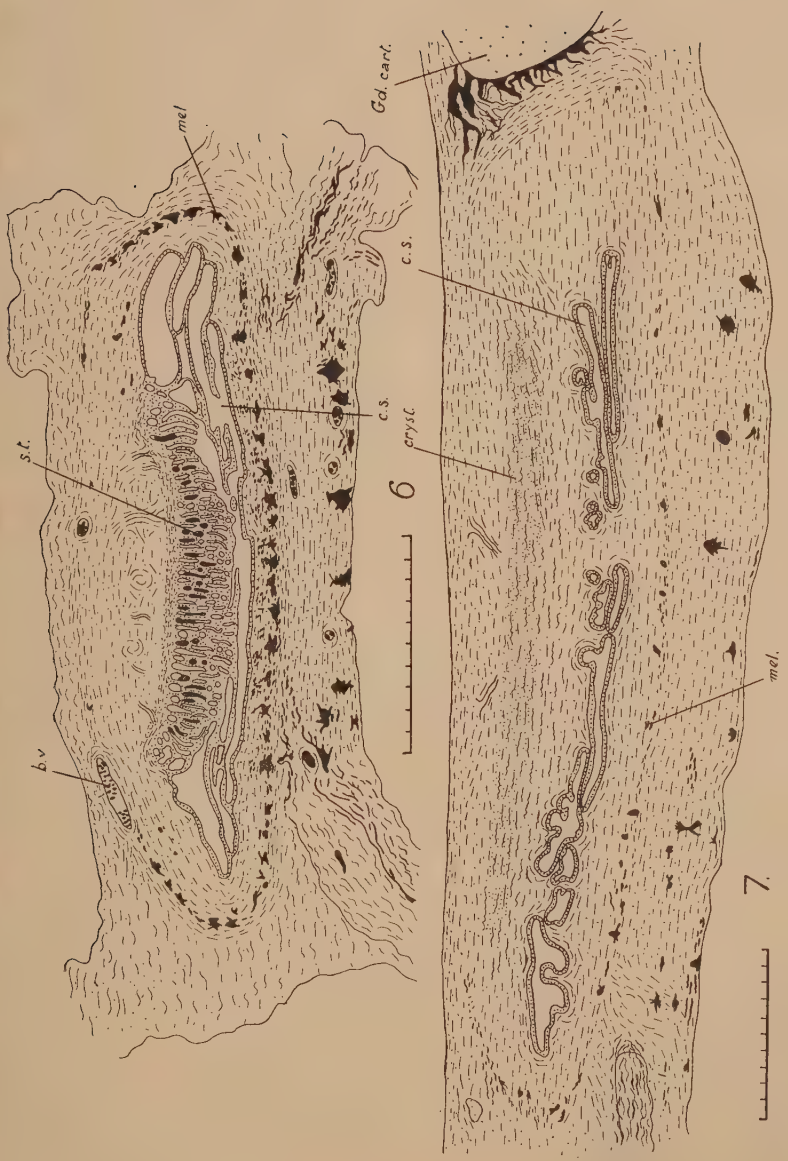
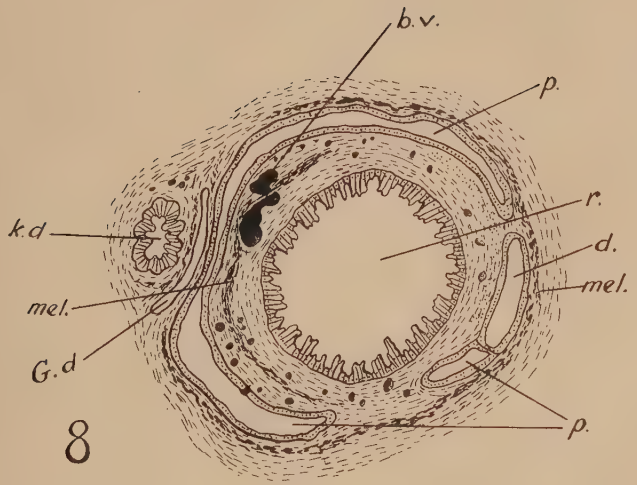

mel
Gd. cart.
c.s.
c.s.
cryst.
s.t.
6
b.v
mel.
7.

PLATE IV.—Sections transverse to the rectum in two specimens of *C. cœlorhynchus*.

FIG. 8.—In a 21-cm. specimen.

FIG. 9.—In a 30-cm. specimen.



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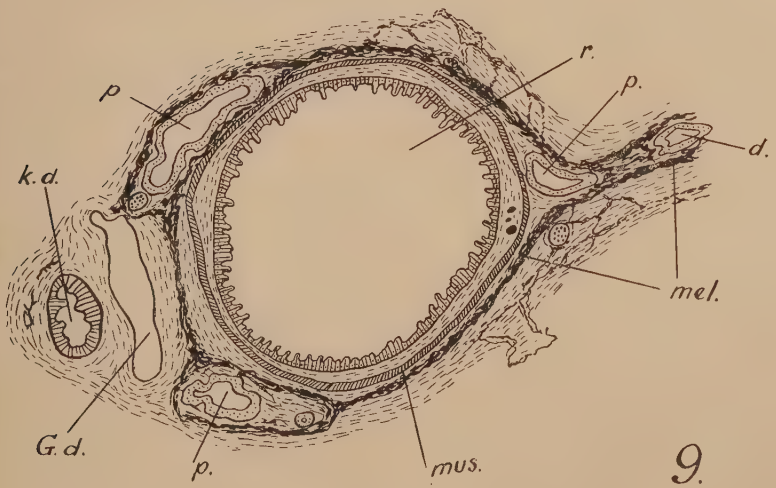

9.

Growth and Maintenance in the Plaice (*P. platessa*, L.) Part II.

By

Ben Dawes, A.R.C.S., D.I.C., F.L.S.

With 11 Figures in the Text.

CONTENTS.

PAGE

Introduction and Methods 877

I. Results of the Cawsand Experiments of 1930 879

1. Minimum Requirements and Maintenance 879

2. Maximum Requirements and Growth 884

3. Growth and Intermediate Food Supplies 891

4. Conclusions drawn from the Experiments, 1930 (Cawsand) . . . 893

II. Results of the Lympstone Experiments of 1930 896

1. Experiments of January to April 896

2. Minimum Requirements and Maintenance 897

3. Maximum Requirements and Growth 899

4. Conclusions drawn from the Experiments of 1930 (Lympstone) . . 902

III. General Discussion of the Results of the Plaice Experiments of 1928–1930 903

IV. Acknowledgement 915

V. Tables 1–66 916–947

INTRODUCTION AND METHODS.

In a previous paper (Journ. Mar. Biol. Assoc., Vol. XVII, No. 1, Sept., 1930) I described in detail a series of experiments on Growth and Maintenance in the Plaice which were carried out during 1928 and 1929 at Cawsand, near Plymouth, and at Lympstone, near Exmouth. The purpose of the present paper is to present and discuss data collected at the above places during 1930 in continuing the experiments. The conditions under which the last experiments were carried out were identical with those of 1929, i.e. refined with reference to those of 1928. Several additions were made, however, to the series of 1929. Two fish of each sex were supplied with maximum rations while living under the conditions maintained during 1928 and were segregated in a box, which had the original $\frac{1}{2}''$ mesh wire-netting windows but which was divided up into compartments. This experiment was arranged in order that we might ascertain the possibility

of food leakage due to tidal action during the course of the earliest experiments, but it also enables us to compare the results obtained under slightly different conditions of water circulation, which is important in view of the fact that the windows of the modified boxes were of necessity minimal in area.

There is yet another departure from the experiments of 1929. The results of the experiments of 1928 tended to show, if in a rather unsatisfactory manner, that fish taking quantities of food intermediate between maintenance and maximum rations made more efficient use of food for purposes of growth than did maximum-fed fish. Unfortunately, the refinement of the preliminary experiments during 1929 entailed an additional amount of routine work and made it impossible to check these promising results. The question of increased efficiency with intermediate-feeding is a very important one, and it was deemed advisable during 1930 to obtain evidence which would either support or refute the conclusion so tentatively put forward and to impart some finality to our views. Accordingly, two fish of each sex were kept segregated under the refined conditions and in parallel with maximum-fed fish. These fish were supplied with intermediate rations, roughly twice the value of maintenance rations. The number of fish is admittedly small, but it must be emphasised that the inclusion of these four fishes in the experiment distended the routine work to such an extent that it was quite impossible to include more.

Apart from these details, the experiments of 1930 were identical with those of 1929. Feeding operations were performed daily, the food being *Mytilus edulis* as before, and especial care was taken to avoid the inclusion of food fragments likely to be washed out of the boxes by the tides. Each day, before food rations were presented to the fish, the surplus food from the previous meal was meticulously removed and "weighed back." Weighing and measuring operations were performed fortnightly as before, and the time interval between the last meal of the fortnight and the weighing was maintained as constant as was practicable. Temperature records were kept at Cawsand and Lympstone alike, graphs of weekly maxima and minima being prepared finally from them.

Before passing on to a description of results, it must be mentioned that during the first two months the routine work was carried out by Miss Thursby Pelham of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft, and her assistant, under my supervision and direction. Again, during early September I was on leave and in mid-September resigned my position in order to take up an appointment in London. The experiments were therefore directly carried out by me only during the latter part of June and during July and August. When I left the work, Messrs. G. M. Spooner and J. E. Smith of the Marine Biological Association Laboratory, Plymouth, jointly took charge of the experiments, the data

of which were sent to me in London. Before proceeding I wish to record my thanks to the above-mentioned people for their contributions to the work. Mr. H. Lees, Tank Superintendent of the Ministry of Agriculture and Fisheries Mussel Tanks, Lympstone, retained his charge of the Lympstone experiments under the direction of Dr. E. S. Russell and Mr. T. Edser, who kindly sent me the data obtained. Thanks are due to Mr. Lees for his share of the work and especially for the observations which were continued throughout the winter of 1929.

I. RESULTS OF THE CAWSAND EXPERIMENTS OF 1930.

1. Minimum Requirements and Maintenance.

Data concerning the experiments with male fish supplied with minimum rations are shown in Tables 1–6, these fish being referred to as C1–C6. It will be seen from these tables that the initial sizes of the C fish form an ascending series from C1 (13·1 cm. ; 21·5 gm.) to C6 (23·0 cm. ; 115·0 gm.), and it will be realised that the size range thus covered by the series is roughly the size range of growth of maximum-fed, fully-growing plaice during the third season of growth. The aim of these experiments is to obtain data which can be applied to an estimation of the gradually increasing maintenance requirements of plaice which are passing through this season of unrestricted growth. The uniformity displayed in the results of earlier experiments of the same kind with numbers of fish of the same size, justifies this attempt to measure the variation in maintenance requirements with single fish of increasing size, particularly as earlier work had not provided really satisfactory results and as there was no possibility of increasing the number of fish included in the experiments.

The smallest fish, C1 (initial length 13·1 cm. ; initial weight 21·5 gm.),* was maintained fairly constant in weight over a period of 144 days (final weight, 20·8 gm.), slight losses during the early part of the experiment being due to a refusal to take food. The total quantity of food taken during 144 days was 66·9 gm., or roughly 3 times the mid-body-weight. During the various fortnightly periods, less than 0·40 gm. of food per day resulted in slight loss of weight ; more than 0·50 gm. per day in increase in weight except in one instance (Sept. 19th, Table 1). The maintenance requirements of a 21 gm. male plaice thus appear to lie between 0·4 gm. and 0·5 gm. of food per day, i.e. between 0·019 and 0·024 of the mid-body weight for the periods considered (=the maintenance ratio). For 144 days, an average of 0·46 gm. of food per day was required, yielding a maintenance ratio of 0·022.

* In the descriptions to follow, the abbreviations init. lth. (for initial length) and init. wt. (for initial weight) will be used.

C2 (init. lth. 15·3 cm.; init. wt. 31·5 gm.) (Table 2) showed slight periodic fluctuations in weight, but after 176 days had increased its initial weight by only 4·4 gm. Slight losses in weight occurred during three periods when the average daily ration was less than 0·47 gm., slight gains during eight periods when it was more than 0·45 gm. The maintenance ration would therefore appear to be 0·45–0·47 gm. per day, the corresponding ratio 0·013–0·014. Over 176 days the average daily ration was 0·49 gm., the maintenance ratio 0·015.

C3 (init. lth. 16·9 cm.; init. wt. 45·0 gm.) (Table 3) was kept to within 2 gm. of its initial weight over a period of 162 days, despite periodic fluctuations. This fish can be taken to illustrate the difficulty entailed in an attempt to maintain the weight of any plaice constant for a succession of fortnightly periods, although it would be easy to find other suitable examples. For the period ending June 23rd the average daily ration was 0·57 gm. and the body-weight was maintained constant. Consequently, a similar average daily ration was supplied during the next following period to July 7th, which resulted, however, in the relatively great weight increase of 1·9 gm. During the next period to July 21st the ration was lowered by 0·03 gm. per day and the result was good, the weight increase being only 0·1 gm. A further slight reduction of the ration (by 0·03 gm.) during the period ending August 6th resulted in an enormous loss of 2·5 gm. in body-weight, and an increased ration (by 0·02 gm.) during the period ending August 21st also resulted in loss of weight (1·2 gm.). Thereafter, much greater daily rations were required to maintain the weight of the fish constant. This succession of incidents is important and has been dealt with at such length because it shows clearly how maintenance requirements seem to fluctuate, a difficulty which must inevitably be encountered in work of this kind. The difficulty is referred to briefly in my previous paper (p. 119).

During the first 100 days of the experiment, C3 lost weight during fortnightly periods whenever less than 0·53 gm. of food was taken on the average per day, and gained weight whenever more than 0·54 gm. was taken, so that these quantities mark the limits of the maintenance requirements, the maintenance ratio being 0·011–0·012. By comparison with the results for other fish, these values are low and this observation is perhaps not altogether disconnected with the fact that beyond this period and to the end of the experiment, C3 required greatly enlarged daily rations for maintenance. It is suggested that during the early part of the experiment weight was maintained constant on unusually small rations by atypic means (e.g. excessive water imbibition), after which some adjustment was effected causing much greater rations to be necessary. For the whole period of 162 days, the average daily ration was 0·64 gm., providing a maintenance ratio of 0·015.

The results of the experiments with the males C4, C5, and C6 are shown in Tables 4, 5, and 6. Taking C4 (init. lth. 18·9 cm. ; init. wt. 59·0 gm.) first, and noting for the various fortnightly periods loss or gain in weight following upon varying average daily rations, it is found that the maintenance requirements appear to be 0·85–0·86 gm. of food per day (maintenance ratio=0·014). During the whole course of the experiment (178 days), an average daily ration of 0·89 gm. was taken and at the end of this period the initial weight had been increased by 3·5 gm., so that the maintenance ratio of 0·015, which is indicated, is slightly high. C5 (init. lth. 21·2 cm. ; init. wt. 86·7 gm.) appeared to require daily rations of from 0·87 gm. to 0·99 gm. for maintenance from one period to another (Table 5), this giving a maintenance ratio of 0·010–0·011. For the whole period of 164 days an average daily ration of 0·95 gm. was taken and the total loss in weight was 1·3 gm., so that for the whole experiment the maintenance ratio was 0·011, which closely follows the figures indicated by study of the fortnightly periods. The largest fish, C6 (init. lth. 23·0 cm. ; init. wt. 115·0 gm.), seemed to require 1·27–1·40 gm. of food per day for maintenance (Table 6), which suggests a maintenance ratio of 0·011–0·012. During 147 days it took an average daily ration of 1·42 gm. and lost a total of 3·9 gm. in weight, so that for this period a maintenance ratio of 0·013 is indicated.

The results of these maintenance experiments with male plaice are summarised for the whole course of the experiments in Table 7. It is seen that the average daily ration required for purposes of maintenance by males of from 13·1 cm. to 23·0 cm. and from 21·5 gm. to 115·0 gm. gradually increased from 0·46 to 1·42 gm., neglecting slight losses in body-weight. The corresponding maintenance ratios gradually decreased from 0·022 to 0·012. The difference between these quantities and values represents the difference between the maintenance requirements of different male plaice of various sizes, but by analogy it can be interpreted as representing the difference between the maintenance requirements of a single male plaice at various times during its third season of unrestricted growth, since the limiting sizes are approximately those which mark the two extremes of this season, having been chosen to serve this purpose. In Figure 1 the decrease in value of the maintenance ratio with increase in size of fish, and therefore by analogy also with increasing age, is shown graphically.

The female plaice used in maintenance experiments, like the corresponding males, were arranged so as to form a series having a total size range comparable with that passed through by a freely-growing female plaice during its third season. The results of these experiments are shown in Tables 8–13, the fish being referred to as A5, A6 (a and b), A2, A4, A3, and A1, and being taken in this order, which is that of increasing size.

In the descriptions to follow the maintenance requirements suggested by the study of fortnightly data will be found, as in the case of males, by noting the periods when loss or gain in weight occurred on average daily rations below or above a certain value. This will render unnecessary much of what would otherwise prove to be tedious and almost endless repetition.

The smallest female A5 (init. lth. 12·2 cm.; init. wt. 17·3 gm) appeared able to maintain itself on average daily rations of 0·39–0·47 gm. for six consecutive fortnightly periods (July 7th–Oct. 6th; Table 8), the

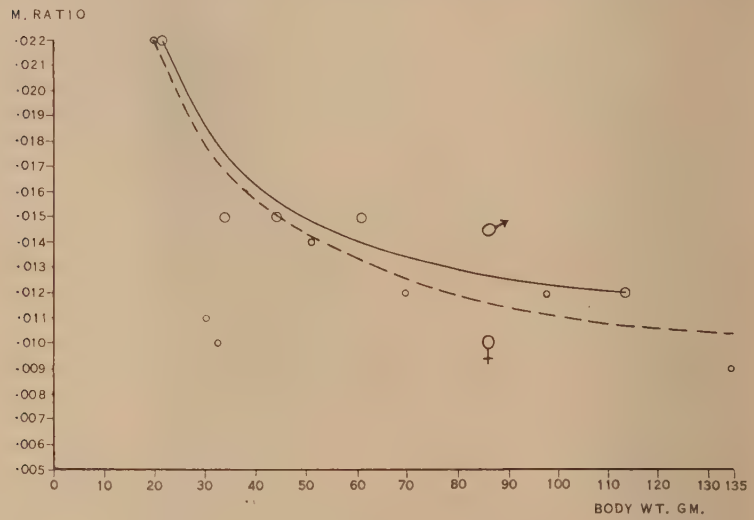

Fig. 1.—Graphs showing, for male and female plaice between 2 and 3 years old, the relationship between the maintenance ratio and the body-weight. Based upon the results of the Cawsand experiments, 1930.

corresponding maintenance ratio ranging from 0·018 to 0·025. During 143 days, an average daily ration of 0·43 gm. enabled the fish to maintain itself and to increase its initial weight by 4·7 gm., so that the maintenance ratio of 0·022 which is thus indicated is slightly high. Both the fish referred to as A6 steadily declined to take food in sufficient quantities to satisfy their maintenance requirements, with the result that they consistently and steadily lost weight. The data for these fish are shown in Table 9 but we must decline to make use of them since they would scarcely assist our purpose. A glance at the two points widely separated from the curve on the left of Figure 1, which represent the maintenance ratios for these fish, will convince one that these data are best disregarded.

The data for A2 (init. lth. 17·3 cm. ; init. wt. 49·0 gm.), which are shown in Table 10, indicate that from one fortnightly period to another between July 7th and September 5th, this fish was able to maintain itself on daily rations of 0·64 gm.–0·67 gm., the corresponding range in the maintenance ratio being 0·012–0·013. During 148 days an average daily ration of 0·69 gm. allowed of an increase in weight of 4·0 gm., so that the maintenance ratio of 0·014 thus indicated is slightly high. A4 (init. lth. 19·8 cm. ; init wt. 71·0 gm.) required a daily ration of from 0·83 gm. to 0·94 gm. for purposes of maintenance (Table 11), showing a maintenance ratio of 0·012–0·014. Over a period of 178 days there was a slight loss of weight (2·7 gm.) after an average daily ration of 0·87 gm. had been taken, this figure indicating a maintenance ratio of 0·012.

A3 and A1 were both initially over 100 gm. in weight. The daily ration required for maintenance by A3 (init. lth. 22·0 cm. ; init. wt. 101·5 gm.) was 1·21–1·29 gm. from period to period (Table 12), the corresponding ratio being 0·012–0·013. During 147 days, an average daily ration of 1·21 gm. resulted in 6·3 gm. loss in weight, so that the maintenance ratio is slightly greater than 0·012. A1 (init. lth. 24·5 cm. ; init. wt. 132·0 gm.) required 1·23 gm.–1·36 gm. of food per day for purposes of maintenance from one period to another, the maintenance ratio being 0·009–0·010. During 178 days an average daily ration of 1·24 gm. allowed this fish to increase its initial weight by 4·9 gm., which indicates that the maintenance ratio was slightly less than 0·009.

Summarised data concerning the females used in these maintenance experiments are shown in Table 14, which is set out parallel with Table 7 for males. It is seen that the average daily ration required over long periods for purposes of maintenance by female plaice ranging in length from 12·2 cm. to 24·5 cm. and in weight from 17·3 gm. to 132·0 gm., increases from 0·43 gm. to 1·24 gm., the corresponding maintenance ratio diminishing from 0·022 to 0·009. This then is approximately the order of the changes which would occur in the maintenance requirements of a female plaice during its third season of growth since, as in the case of males, the sizes of these maintained fish were arranged in a series, the size limits of which are also the limits for this season of growth.

Thus for male and female plaice alike, the maintenance ratio at the end of the third season of growth is only approximately one-half its value at the commencement of the season. And this is strikingly shown in another way. The following table shows the multiple of the mid-body-weight taken by male and female plaice during the whole period of experiment indicated in days.

♂Fish No.	No. of Days.	Multiple of Body-wt. of Food (gm.).	♀Fish No.	No. of Days.	Multiple of Body-wt. of Food (gm.).
C1	144	3·2	A5	143	3·2
C2	176	2·5	A6	—	—
C3	162	2·4	A2	148	2·0
C4	178	2·6	A4	178	2·2
C5	164	1·8	A3	147	1·8
C6	147	1·8	A1	178	1·6

The smallest fish is shown at the top of the table, the largest at the bottom, and it is clearly indicated that for the smallest plaice the ratio of food-weight to body-weight is double that for the largest plaice. This is in accordance with the suggestion tentatively put forward in my previous paper, although the method then employed of combining Cawsand and Lympstone data was not altogether legitimate.

2. Maximum Requirements and Growth.

The growth experiments were marked out into two sets, the first consisting of six fish of each sex housed in the modified boxes with fine mesh windows, the second of two fish of each sex confined in boxes with $\frac{1}{2}''$ mesh windows similar to those used in the preliminary experiments. All fish were segregated, however, and were tended separately. The fish kept under the refined conditions will be referred to as D (♂) and B (♀) fish respectively, those kept in boxes with $\frac{1}{2}''$ mesh windows as M (♂ & ♀) fish.

The results of the experiments with D and B fish are summarised in Table 15, where it is seen that considerable variation in rate of growth characterised both groups. Amongst males, the greatest total increases in length and weight were shown by D1 (4·2 cm. ; 41·3 gm.), the least being shown by D6. The rate of growth was generally very slow as compared with that shown during previous years, D1 and D2 succeeding in doubling their initial weights over long periods (162–178 days), other males failing to achieve this result during periods up to 116 days in extent. The relatively slow rate of growth is best shown by comparison of these results with that for D3 during 1929 which, during 175 days, multiplied its initial weight by 6·8. This feature of rate of growth was shown also by females during 1930, none of which achieved much more than a mere doubling of the initial weight, B6 failing to do this, even over a period of 131 days. For the whole range of the experiments the average daily ration varied, among males from 1·6 to 3·0 gm., among females from 1·8 to 3·5 gm., so that appetite was rather smaller than during the previous year, when for corresponding periods it ranged among males from 2·2 to

3·3 gm. and among females from 2·3–4·1 gm., neglecting obviously unhappy fish. Efficiency, i.e. the relation between food taken and growth ensuing, also varied in different fish, but to a slighter degree than during the previous year and it was generally much lower, i.e. the indices were greater. Among males 9·1–11·9 gm. of food was required to produce 1·0 gm. increase in body-weight, among females 8·5–12·9 gm. From the above it will be seen that at Cawsand the growth performances of 1930 were distinctly inferior to those of 1929.

Data concerning the experiment with D1 are shown in Table 16, where it is seen that the increments of length and weight added from period to period were most irregular, especially the latter, which varied from −0·3 gm. to 10·2 gm. per fortnightly period. The average quantity of food taken daily varied similarly during different periods, but only slightly, from 2·15 gm. to 4·08 gm. Variation also occurred in respect to efficiency, as measured by the quantity of food required to produce 1·0 gm. of fish irrespective of time, and was very strongly marked, as will be seen by inspection of col. 6 of the table. The maximum efficiency shown during the whole course of the experiments is represented by the index 4·2 for the period ending June 9th.

Similar results were shown by the other male plaice, D2–D6 (Tables 17–21). In the case of D2, growth in weight per period varied from −0·9 gm. to 7·0 gm., the average daily ration taken from 1·00 gm. to 2·64 gm., and efficiency between tremendously wide limits (Table 17). Efficiency was greatest (index 4·3) during the period of maximum growth (June 9th ; 7·0 gm.). D3 showed maximum appetite (3·20 gm. per day), maximum growth (10·6 gm.), and maximum efficiency (index 4·2) for the whole experiment during the period ending July 21st when the ratio W/L^3 attained its maximum value (0·011), (Table 18). D4 shows the maximum increase in weight for any period (6·2 gm.) when the appetite was a maximum (2·2 gm. per day) during the period ending August 6th, when efficiency was high (index 5·7) but not a maximum (period ending June 9th ; index 4·4). At the same time W/L^3 reached its maximum value of 0·010 (Table 19). D5 and D6 did not show such high degrees of efficiency at any time, the maxima for these fishes being represented by the indices 6·5 and 7·1 (Tables 20 and 21).

The results of the experiments with males thus lend support to the conclusion previously put forward that there is no simple relationship between the quantity of food taken and the degree of growth ensuing. They also show that such relatively high efficiency as is indicated by the indices 4·2–4·4, which neglect maintenance allowances, may be attained at certain indeterminate times during the growth season. In Figure 2 the results of these experiments are shown graphically as regards growth increments of weight. It will be seen that the slopes of the curves shown

are much less steep than that of the typical sigmoid curve shown for D3 (1929) in my previous paper (Fig. 5, p. 123).

Data concerning the female B1 are shown in Table 22. Growth in length per period varied from −0·1 cm. to 0·9 cm., growth in weight from −5·9 gm. to 8·6 gm. Growth per period was a maximum during the period ending July 21st, when efficiency was also a maximum (index 4·7) and when the ratio W/L^3 attained its maximum value of 0·011. When

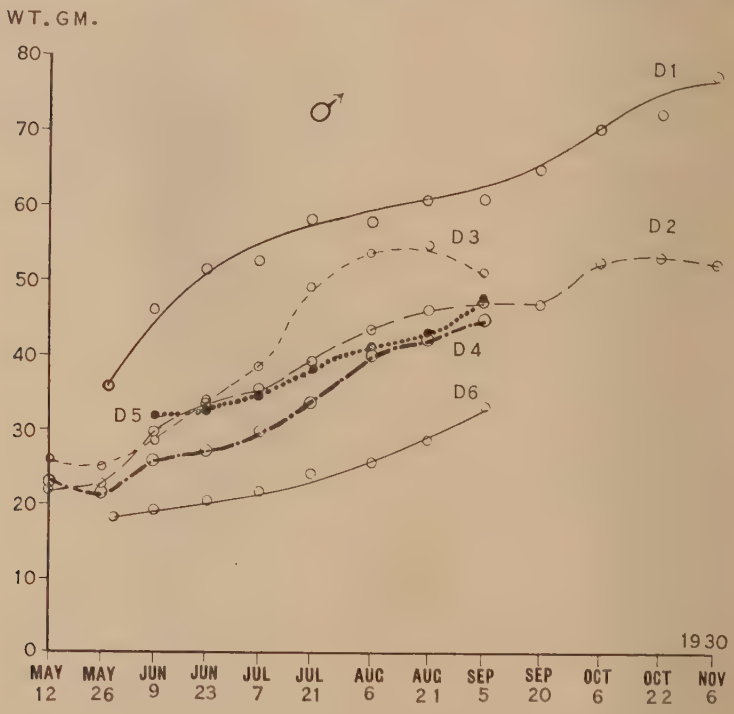

Fig. 2.—Growth curves of weight for the male plaice D1–D6 (Cawsand, 1930).

appetite was greatest (3·48 gm. per day, Aug. 21st), growth and efficiency were both high in degree (7·5 gm. per period; index 7·0). This fish showed consistently high efficiency from June 17th to August 21st, the index 4·7 occurring during two periods, yet as in the case of the males, considerable variation was shown from time to time.

The remaining females showed similar variations in rate of growth, in efficiency and in appetite from period to period, as will readily be seen if Tables 23–27 be scrutinised. B2 took daily rations ranging from 1·32 gm. to 3·56 gm. (neglecting the first week of mere maintenance), increased

in weight by quantities varying from 0·2 gm. to 10·3 gm. per period and showed efficiency indices as widely separated as 4·8 and 30·8 from June to September (Table 23). It is interesting to note that growth, appetite, and efficiency were all maxima during the period ending July 21st. B3 showed maximum growth and efficiency (5·9 gm. ; index 5·4) during the period ending June 23rd (Table 24). B5 was the most promising fish in the experiments until September 5th when it was lost. From Table 25 it will be seen that the periodic increases in length and weight were consistently great, maxima at 1·0 cm. and 11·0 gm. respectively, and that for the whole experiment the efficiency indices lay between the limits 5·5 and 9·2. B4 also showed high efficiency, the index remaining between such narrow limits as 5·1–5·7 for three consecutive periods (Table 26). B6 was the least efficient of the Cawsand fish, the maximum being represented by the index 6·0 (Table 27).

The results of the experiments with females thus confirm completely the conclusions mentioned above regarding the variation in rate of growth, in appetite and in efficiency from period to period during the growth season. The results show also that the maximum values of these three sets of growth characteristics may or may not coincide in time. The greatest efficiencies shown by the females read as follows : 4·7 (B1), 4·8 (B2), 5·1 (B4), 5·4 (B3), 5·5 (B5), and 6·0 (B6), so that the females are slightly inferior to the males in this respect, the efficiencies of these latter reading 4·2 (D1 and D3), 4·3 (D2), 4·4 (D4), 6·5 (D5), and 7·1 (D6). That such efficiency is not sustained will readily be seen by examination of the tables mentioned above.

The growth curves of weight for the females (Fig. 3) are seen to compare fairly closely with those shown in Fig. 2 for the males. The slopes of the curves are much less steep than those of the curves for B1 and B4 (1929) as shown in Fig. 7 of my previous paper (p. 125).

Turning now to the results of the experiments with M fish (maximum-fed fish housed in boxes with $\frac{1}{2}''$ mesh windows), we see in Table 28, where these results are summarised, that growth has proceeded at much the same rate as in the case of the D and B fishes, excepting M1, which surpassed the rest in performance and succeeded in tripling its initial weight, M2, M3, and M4 failing to achieve this result. The quantities of food taken by this fish greatly exceeded those taken by the D and B fish, but efficiency was not as high. These values, however, are affected by certain incidents to be mentioned below, where it is shown that these results do not have the same degree of accuracy that the other results have.

During the preliminary experiments of 1928, fish supplied with intermediate rations appeared to show a greater efficiency in utilising food for purposes of growth than fully-feeding fish. But intermediate-fed fish invariably take the whole of the ration supplied to them whereas in the

case of maximum-fed fish there is a distinct possibility of food remaining over from a meal. If during 1928, small quantities of food remained in the boxes containing the latter fish and were washed out of the boxes by ebbing or running tides, then the observed efficiency of the maximum-fed fish would be lower than the true efficiency. By comparison, the efficiencies of intermediate-fed fish would appear higher. During the earliest experiments there was no sign of appreciable quantities of food

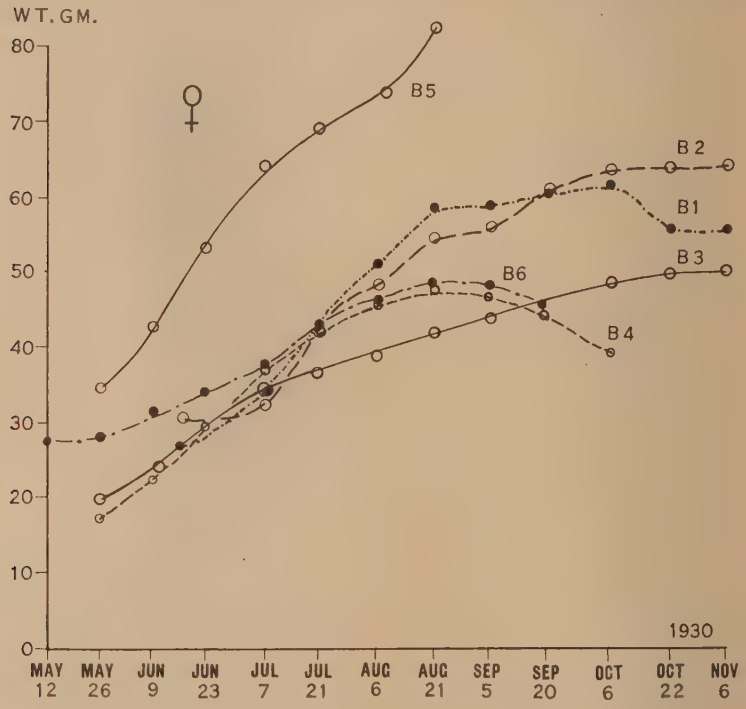

Fig. 3.—Growth curves of weight for the female plaice B1–B6 (Cawsand, 1930).

left over from meals. That fact in itself is strange, since later experiments have shown conclusively that in fish as in other animals, appetite is by no means constant. Thousands of observations made since 1928 have proved this point. Hence it was considered essential to apply a series of tests in order to ascertain the possibility of food having been swept out of the boxes during the earliest experiments. The matter concerns not only food loss but also efficiency of maximum-fed fish, and more importantly of intermediate-fed fish.

To test this possibility of food leakage, Miss Thursby Pelham was asked

to place into the boxes containing M fish quantities of food greatly in excess of the normal ration for such fish and to weigh back the quantities remaining on the next day. This was done during the period ending July 7th and by myself during that ending July 21st. More than this, the wire-netting windows were alternately cleared of epifauna and epiflora (as they were regularly during 1928) and allowed to foul (which may happen in one to a few days).

That the data from these tests were conclusive is seen in the single case of M1. During the last seven days of the period ending June 23rd, this fish was taking a ration of less than 6·0 gm. per day when the box windows were slightly fouled. During the period ending July 7th, it was supplied with 15·0 gm. of food on each of eight days, when the windows were cleared, and no food was found to remain. In some of the tests when this quantity of food was presented, varying quantities were left over, but the essential point is that on many days, often consecutively, no food remained although much more than a normal ration had been presented and despite the fact that sea conditions were calm. In the tests I applied myself this was similarly the case. Consequently, henceforth I used my own judgment as to the constitution of maximum rations and for the rest of the experiment provided what was considered to be an adequate but not overadequate ration, thus ensuring that loss should be minimal if and when it did occur. This applies only to the M fish of course.

In view of the above-mentioned facts it appears reasonably certain that during 1928 there was some loss of food, yet it must be emphasised that the quantities supplied during this year were not unduly large. Moreover, the fish were active and showed a rapid growth-rate. They took food avidly and it is likely that the losses were small. But loss undoubtedly must have occurred. Hence the suggestion of increased efficiency on the part of intermediate-fed fish is open to doubt, or at least objection. This question, however, has been set quite independently during 1930 and data of greater reliability have been forthcoming. These will be presented when the results of the experiments with M fish have been described.

Data concerning the M males are shown in Tables 29 and 30 and growth-curves of weight are presented in Figure 4. M1 was the most rapidly growing fish in the experiments, but, like other fish, it showed great variation in rate from one fortnightly period to another. Periods of rapid growth-rate, July 7th, 10·3 gm.; August 21st, 11·0 gm.; October 6th, 12·6 gm., and November 6th, 15·7 gm., alternated with periods of slow rate, June 23rd, 0·9 gm.; July 21st, 4·0 gm., and October 23rd, −0·2 gm., growth in length paralleling growth in weight (Table 29). Food rations were much more regular than weight increases, so that efficiency must have varied widely from time to time. Similar general results hold good for the male

M2, which, however, showed increasing degrees of growth, appetite and efficiency as the experiment proceeded (Table 30).

The results for the females M3 and M4 are very similar to those for the

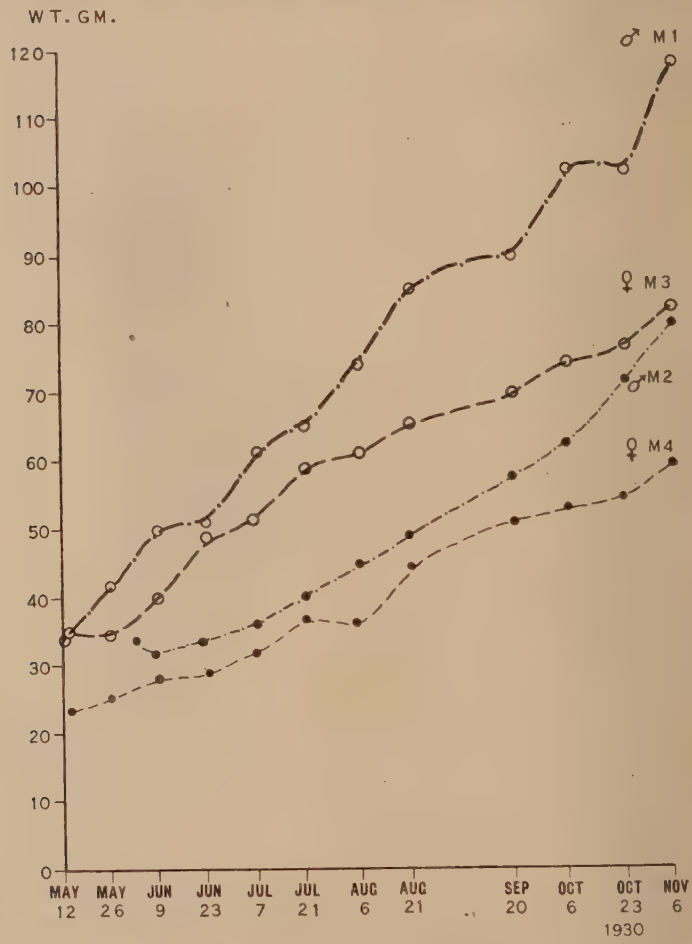

FIG. 4.—Growth curves of weight for the male plaice M1 and M2 and for the female plaice M3 and M4 (Cawsand, 1930).

males. Considerable differences are shown in the degree of growth, appetite, and efficiency occurring during different periods. These results are shown in detail in Tables 31 and 32.

It is evident from the foregoing description that the M fish do not display a rate of growth markedly different from that of the D and B fish, so

that we are able to assert that the conditions of life in the boxes containing the former fish are not markedly superior to those containing the latter, which are the modified boxes with reduced windows of minimum-sized mesh. In maximum-fed fish generally, the growth-rate during 1930 was slower than during 1929, no matter whether we consider the experiments with D and B fish, or those with M fish of the latter year. This was doubtless due to factors other than those arising out of the methods of experimenting and the type of accommodation afforded the fish.

3. Growth and Intermediate Food Supplies.

The results of the experiments with M fish indicate the necessity for looking beyond the experiments of 1928 for a satisfactory answer to the question as to whether intermediate-fed fish utilise food more efficiently than do maximum-fed fish. Fortunately, this question was investigated as a separate concern during 1930. Two fish of each sex were kept segregated in one of the modified boxes and were supplied with rations intermediate in quantity between maintenance and maximum rations, and roughly twice the value of the former. Apart from the size of rations, these fishes were treated in exactly the same manner as maximum-fed fish and lived under similar conditions, so that it is perfectly legitimate to compare the rate of growth and degree of efficiency with the corresponding characteristics in the case of D and B fish. In the description which is to follow, these fish will be referred to as I fish, I1 and I2 being males, I3 and I4 females.

The summarised results of these experiments are shown in Table 33, where it is seen that for periods of 141–178 days, an average daily ration of not more than 1·5 gm. was provided. In the cases of the D and B fish as much as 3·5 gm. per day was taken. It is seen also that for the whole range of the experiments the I fish showed an unmistakably higher degree of efficiency than was shown by the D and B fish. No fish of the latter categories approached an efficiency such as is indicated by the indices 6·8, 6·3, and 5·9 for I1, I3, and I4 respectively. I2 showed relatively high efficiency (index 10·5), which was surpassed, however, by that of D4, B3, and B5 (indices 9·1, 9·7, and 8·5 respectively).

The detailed results of the experiments are presented in Tables 34–37. In the first of these, it is seen that I1 showed consistently high efficiency from June 13th to October 6th, the greatest index during this period of 98 days being 8·6, while during each of four periods the index was less than 5·0. By way of contrast it might be pointed out that during the whole series of experiments with six D fish, the index of efficiency was smaller than 5·0 during a total of only four periods. I1 also showed a rapid growth-rate, and almost doubled its initial weight of 33·7 gm. over a period of 146 days. I2, the remaining male, was not a particularly

efficiently growing fish, but it showed an efficiency index smaller than 9·0 on four occasions, neglecting the index for the period ending June 23rd. This last index is extraordinarily small and perhaps of doubtful accuracy, there being the possibility of weighing error during the previous period, although length records suggest that this view is not fully justified (Table 35). Fusing the two periods between May 26th and June 23rd yields an index of 5·4 for the month, showing that in any case this fish showed a very high degree of efficiency about this time.

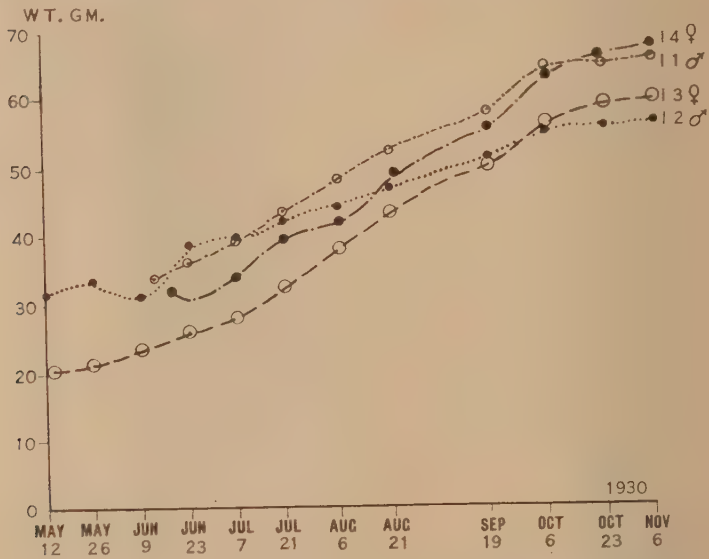

FIG. 5.—Growth curves of weight for the male plaice I1 and I2 and for the female plaice I3 and I4, fish supplied with intermediate rations (Cawsand, 1930).

Turning to the females we find in I3 a most surprisingly efficient fish. In Table 36 it is seen that during one period only 3·8 gm. of food were required to produce 1·0 gm. increase in weight (July 21st). During three other periods, the efficiency index was smaller than 5·0 and during three others it was less than 6·0. It is readily seen to be far more efficient than any one of the B fish. During 176 days the initial weight of this fish was almost tripled (Tables 33 and 36). I4 showed more variable efficiency, which however was generally distinctly high. Table 37 shows that the efficiency index was 3·1 during one period, 4·5 or smaller during three others, and the largest index for the whole experiment was 10·8. This fish easily doubled its initial weight over a period of 141 days (Table 33).

Examination of the data of these experiments on intermediate feeding shows that whether we consider efficiency over periods of 141–178 days or for much shorter periods, there is considerable justification for the conclusion that male and female plaice alike make relatively much more efficient use of food for purposes of growth when supplied with rations intermediate between their maintenance requirements and maximum demands and roughly twice the value of the former than when maximum quantities of food are supplied to them. Among such intermediate-fed fish, not only are efficiency indices of 4·0–5·0 more common, but there is also less variation in efficiency, the larger figures met with among the

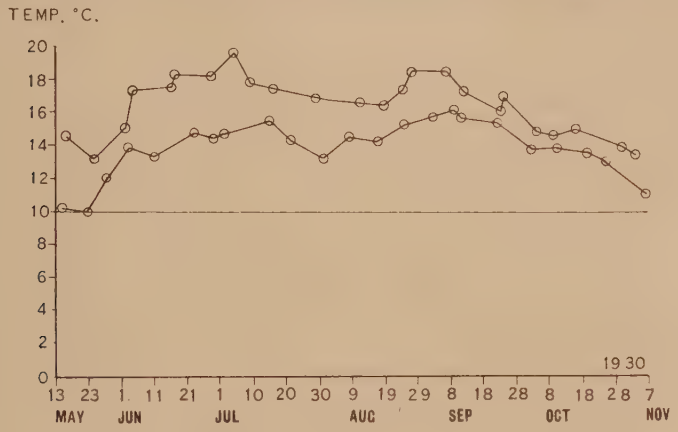

Fig. 6.—Graph of maximum and minimum temperatures at Cawsand, 1930.

indices for maximum-fed fish being absent. In the case of the four plaice we have considered, only during 12 periods out of a total of 40 was the efficiency index greater than 10·0, which fact in itself is much more than merely a suggestion of consistently high efficiency. The growth curves of weight for these fishes are presented in Figure 5.

Figure 6 shows graphically the temperature range at Cawsand during 1930, minimum and maximum temperatures being plotted against time.

4. Conclusions Drawn from the Experiments of 1930 (Cawsand).

(1) A male plaice 13·1 cm. in length and 21·5 gm. in weight was able to maintain its weight to within 0·7 gm. of constancy over a period of 144 days from May 14th, 1930, on an average daily ration of 0·46 gm. of Mytilus flesh, which gives a maintenance ratio, i.e. the ratio food consumed per day/mid-body weight, of 0·022. These values would hold good for a fish just commencing its third season of growth.

(2) Increasingly large fish require larger daily rations for purposes of maintenance, but increase in size of these rations is not directly proportional to increase in body-weight. Experiments with a graded series of fish of various sizes characterising the third season of growth show that the maintenance ratio decreases with increase in size of fish. Thus, passing to the upper limit of the series, a male plaice 23·0 cm. in length and 115·0 gm. in weight was able to maintain its weight to within 4·0 gm. of constancy over a period of 147 days from June 12th, 1930, on an average daily ration of 1·42 gm. of similar food, thus showing a maintenance ratio of 0·012. These values would hold good for a male fish just ending its third season of growth.

(3) The values given above vary from one fortnightly period to another throughout the year, but it is suggested that a reasonably accurate estimate of the increasing maintenance requirements of a freely-growing, two-year-old male plaice would be afforded by accepting the averages for long periods such as the above, excluding the winter months of low temperatures. Thus the maintenance requirements of such a fish would vary between 0·46 gm. and 1·42 gm. of Mytilus flesh per day, the maintenance ratio decreasing from 0·022 to 0·012, approximately. Intermediate values are presented in the text and, graphically, in Figure 1.

(4) A female plaice 12·2 cm. in length and 17·3 gm. in weight was able to maintain its weight to within 4·7 gm. of constancy over a period of 143 days from June 16th, 1930, on an average daily ration of 0·43 gm. of similar food, thus showing a maintenance ratio of 0·022. Thus the maintenance requirements of male and female plaice just commencing the third season of growth appear to be almost if not quite identical.

(5) Among females, as among males, the maintenance ratio decreases with increase in size of fish. Thus, passing to the upper limit of a graded series of fish chosen to represent stages in the third season of growth, a female plaice, 24·5 cm. in length and 132·0 gm. in weight, was able to maintain its weight to within 4·9 gm. of constancy over a period of 178 days from May 12th, 1930, on an average daily ration of 1·24 gm. This value would hold good, approximately, for a fish just ending its third year of growth.

(6) From (4) and (5) it may reasonably be assumed that the maintenance requirements of a freely-growing female plaice would vary, during the third season of growth, between approximately 0·43 gm. and 1·24 gm. of Mytilus flesh per day, if growth were as unrestricted as for example at Cawsand during 1928. The main-

tenance ratio would vary between 0·022 and 0·009 approximately, steadily falling to the latter value. Intermediate values are presented in the text and, graphically, in Figure 1.

(7) Growth of maximum-feeding plaice at Cawsand was poor during 1930 and the rate of growth was much more irregular than it was during 1929. But the results of the experiments with such fish lend support to the conclusions put forward earlier, that growth and efficiency (as indicated by the quantity of food required to produce 1·0 gm. increase in fish weight) vary very considerably in both males and females, from one fortnight to another throughout the growth season. During certain fortnightly periods, but rarely, such high efficiency as is shown by the indices 4·7 (B1), 4·8 (B2), 4·2 (D1 and D3), 4·3 (D2), and 4·4 (D4) was indicated, but during many periods the efficiency shown was very much lower. Thus among males, during 30 periods out of a total of 52, more than 10·0 gm. of food was required to bring about an increase in weight of 1·0 gm., and during 13 of these, more than 15·0 gm. was required. Among females more than 10·0 gm. was required during 26 periods out of a total of 54 ; during 18 of these, more than 15·0 gm. was required.

(8) It has been shown that during the experiments of 1928, there was possibly some leakage of food materials through the coarse meshwork of the box windows, and it is concluded that the efficiency of maximum-feeding fish was higher during this year than the experimental results indicate. This finding also affects the conclusion previously ventured that intermediate-feeding fish show a greater efficiency in the utilisation of food for purposes of growth than maximum-feeding fish. But this matter was investigated independently during 1930, the conclusions formed being shown below (9).

(9) Evidences have been presented which support the conclusion that male and female plaice alike, when supplied with rations intermediate in value between maintenance and maximum rations and about twice the value of the former, utilise food more efficiently for purposes of growth than fish which receive maximum rations. For periods of 141–176 days, the efficiency indices of three fish fed in this way were 6·8 (I1, ♂), 6·3 (I3, ♀), and 5·9 (I4, ♀). No maximum feeding plaice show such consistently high efficiency as this, the smallest indices for a corresponding period during 1930 in the case of such fish were 9·1 (D4, ♂) and 8·5 (B5, ♀). Moreover, indices, for fortnightly periods, of less than 5·0 were much more common among intermediate-feeding fish, 13 out of a possible total of 40 as against 7 out of a possible 106 for maximum-feeding fish. Similarly

for indices less than 10·0, 28 out of a possible 40 as against 48 out of a possible 106. Stated slightly differently, intermediate-feeding plaice required more than 10·0 gm. of food for the production of 1·0 gm. of fish doing only 12 periods out of 40, maximum-feeding plaice during 58 periods out of 106. In the light of this evidence the conclusion of consistently higher efficiency seems perfectly justified.

II. RESULTS OF THE LYMPSTONE EXPERIMENTS OF 1930.

1. Experiments of January to April.

These experiments were really a direct continuation of the experiments of 1929, and thanks are due to Mr. Lees for facing the unpleasantness of winter in continuing the routine observations. As a glance at my previous paper will show, the experiments of 1929 concerned four maintained fish of each sex and four fully-growing fish of each sex. Of these, two of each sex in each group were retained for the work to be described presently, namely, L1 and L4 (♂), and L5 and L7 (♀) of the maintained groups, L10 and L12 (♂) and L13 and L15 (♀) of the freely-growing groups. All these fish were supplied with maintenance rations until summer feeding commenced, when the four latter fish were supplied with full rations. But as long as they declined food, only very small rations were offered so as to reduce the possibility of error, these rations being steadily increased according to appetite as soon as the fish showed signs of taking food readily. The results of these experiments will be described as briefly as possible, but they may be readily grasped by reference to the tables cited.

Maintenance requirements will be indicated by noting rations which result in loss of weight or allow of increase in weight and by considering that these quantities mark the experimental limits of maintenance.

L1w (init. lth. 17·6 cm.; init. wt. 52·8 gm.) during January and February required 0·44 0·61 gm. of food per day for maintenance (Table 38), the maintenance ratio corresponding to these figures being 0·009 0·012. During later months the value of the ratio rose to 0·014–0·015. L4w (init. lth. 21·1 cm.; init. wt. 78·8 gm.) during the first 42 days required a daily ration of 0·77 0·78 gm., the maintenance ratio being 0·010. During the latter part of the experiment these quantities had risen to 1·10 gm. and 0·014 (Table 39). The female L5w (init. lth. 18·9 cm.; init. wt. 69·8 gm.) took rather more food than was required for pure maintenance and consequently it is more difficult to fix maintenance requirements. But during the early stages of the experiment 0·43 0·64 gm. seemed to be the daily ration required, giving a ratio of 0·006 0·009 (Table 40). L7w (init. lth. 22·7 cm.; init. wt. 103·4 gm.) took rather less food than it needed for maintenance and declined

in weight. During the colder months the maintenance requirements were 0·64–0·96 gm. of food daily, which gives a ratio of 0·006–0·009 (Table 41).

In Table 42, the above results are summarised for the whole period of 112 days and included in the table are data from my previous paper showing the maintenance requirements of the same fishes during 1929 to November 25th, so that the decrease in value of the maintenance rations can readily be seen. In the first example, that of L1, the daily ration required for purposes of maintenance fell from 0·90 gm. to 0·58 gm. during the colder months, the maintenance ratio from 0·017 0·011. Other examples will be found in the table.

The plaice which had been maximum-feeding and freely-growing previously, ceased to feed during early November, as is shown in my previous paper, and immediately commenced to lose weight. Having ceased to take maximum rations, these fish characteristically refused even mere maintenance rations. Thus during 56 days from January 6th, it is doubtful if L10w–L15w took food at all (Table 43). On the other hand, minimum-feeding fish continued to take food throughout the colder months, during 56 days from January 6th, the average daily rations taken by L1w–L7w ranging from 0·61–0·91 gm. (Table 43). It is interesting to note also that the losses in weight shown by these non-feeding fishes were not very much greater than those of imperfectly maintained fish (see table). This is the most striking feature of the winter experiments.

The data of the experiments with previously maximum-feeding fish are presented in Tables 44–47. In all cases, it will be seen, feeding has been resumed completely before the end of March. In every case, little more than a maintenance ration was taken daily from February 17th to March 3rd. During the next fortnight the daily ration accepted increased until by March 17th the fish were almost fully-feeding. These findings present no new knowledge, but they do tend to show that the experimental fish behaved in very much the same way as do fish living under natural conditions, which speaks well for the experimental conditions.

When feeding was resumed, the fish at once showed increases in weight and corresponding to these, remarkably high degrees of efficiency, especially during the first fortnight of partially-resumed feeding. [Note : the efficiency index of L15 (Table 47) is misleading, since weight increases are undoubtedly due to ripening of the gonads.]

2. Minimum Requirements and Maintenance.

The results of the experiments on maintenance in male plaice are shown in Tables 48–51, the fish being referred to as L1–L4 respectively. These fish were much larger than the corresponding ones at Cawsand and their maintenance requirements would appear to be those of a fully-growing

plaice at about the middle of its third season, in the larger ones towards the end of this season.

The maintenance requirements of L1 (init. lth. 17·9 cm.; init. wt. 62·2 gm.) lay between 0·71 gm. and 0·98 gm. of food per day from one fortnightly period to another, giving a maintenance ratio of 0·012–0·017 (Table 48). For the whole experiment (182 days) an average daily ration of 0·86 gm. was slightly too small to maintain the weight constant, the corresponding ratio, 0·015, being slightly low (Table 52). For L2 (init. lth. 20·0 cm.; init. wt. 65·0 gm.) the daily maintenance ration lay between 0·83 gm. and 1–14 gm., the ratio between 0·012 and 0·019 (Table 49). Over a period of 182 days an average daily ration of 1·03 gm. allowed of a slight increase in weight, so that the ratio 0·015 is a trifle high (Table 52). L3 (init. lth. 21·5 cm.; init. wt. 90·3 gm.) consistently lost weight to a great extent although the average daily ration taken over a period of 182 days was 1·09 gm., the maintenance ratio 0·013, both of which figures are high by comparison with those for C5 (cp. Tables 50 and 5). L4a (init, lth. 18·5 cm.; init. wt. 65·5 gm.) required 0·82 gm. of food per day for maintenance, the corresponding ratio being 0·013 (Table 51, July 7th and 21st). L4b (init. lth. 24·4 cm.; init. wt. 149·6 gm.) was undoubtedly a three-year-old fish. It required a daily ration of 0·87 gm. for maintenance during the fortnight ending November 10th, a very low value indeed, giving a ratio of 0·006 (Table 51). These results are summarised to November 10th in Table 52, where it is seen that the requirements of L1 alone compare closely with those of Cawsand males (Table 7).

Data concerning the experiments with the females L5–L8 are shown in Tables 53–56. L5 (init. lth. 14·7 cm.; init. wt. 28·9 gm.) showed exceptionally high maintenance requirements (0·80–0·82 gm. per day) (Table 53). Over a period of 182 days an average daily ration of 0·78 gm. was required to maintain the weight constant, the maintenance ratio being as great as 0·027 (Table 57). The data for the remaining females are not of particular interest, and examination of the tables cited will show that the Lympstone results are far less satisfactory and useful than the results of the corresponding experiments at Cawsand. In fact, this statement might be extended to the Lympstone results in general as regards the maintenance experiments. The variations in quantity of the maintenance requirements of the Lympstone plaice are exceptionally wide and the results when examined closely are decidedly discordant. Losses in weight of rather serious proportions were more common than at Cawsand and these detract from the value of the results. Yet it must be remembered that it is not invariably as easy as one might assume to maintain the weights of segregated fish constant since they often decline to take food.

3. Maximum Requirements and Growth.

Summarised data derived from the results of the Lympstone experiments with maximum-feeding plaice are presented in Table 58. The fish, it will be noticed, are generally much larger than those used at Cawsand and most of them are, in all probability, three and not two years old at the

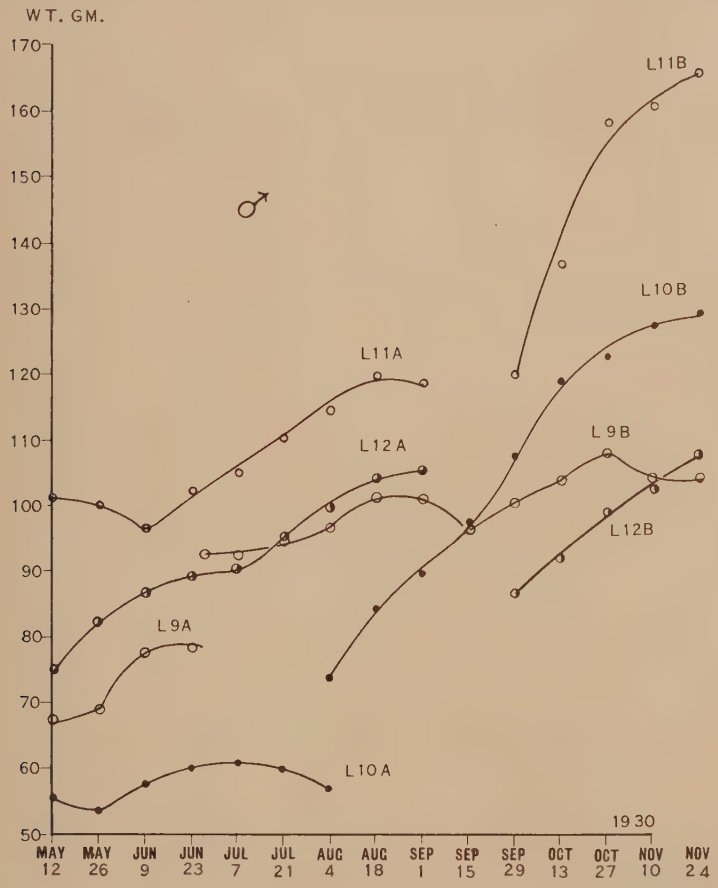

Fig. 7.—Growth curves of weight for the male plaice L9–L12 (Lympstone, 1930).

outset. Such data should provide a useful supplement to those of Cawsand. Unfortunately, in all cases save two the continuity of the experiments was broken, and it was necessary to employ two fishes instead of a single one. The growth-curves of weight for these fishes are shown in Fig. 7.

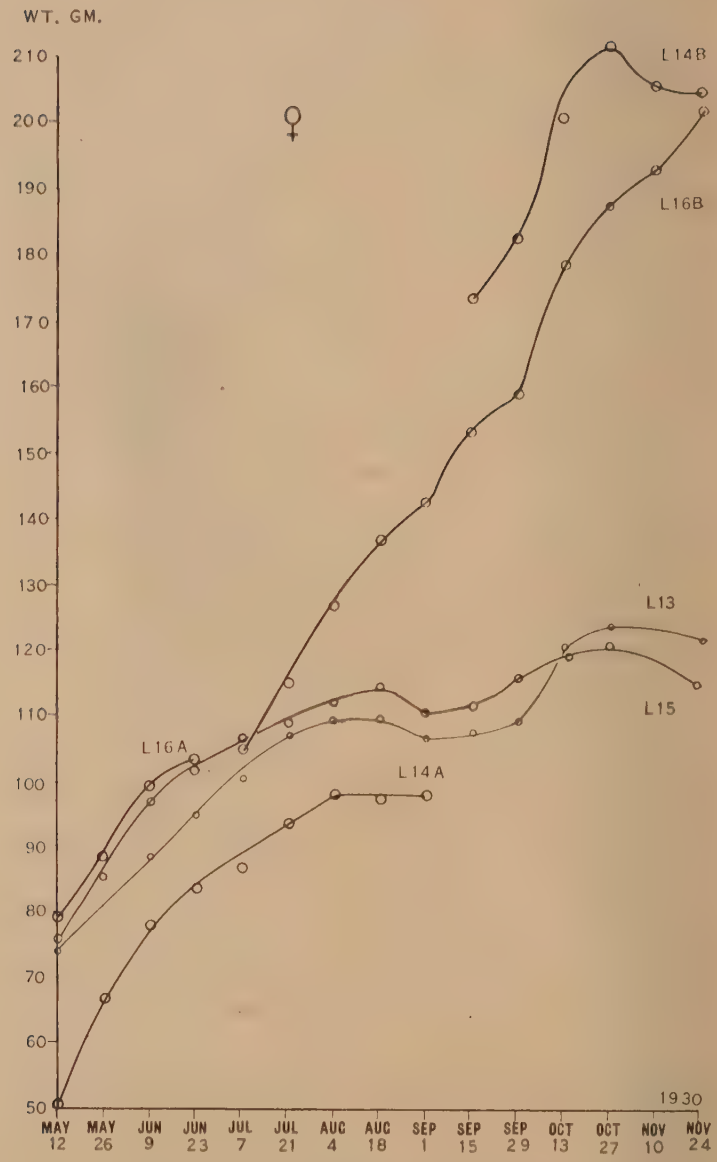

FIG. 8.—Growth curves of weight for the female plaice L13–L16 (Lympstone, 1930).

It is seen from Table 58 that the growth rates of certain fishes were abnormally slow, e.g. those of L9b, L10a, and L11a. In these cases the efficiency indices were tremendously high, 45·4, 186·1, and 26·6 for periods of 84 or more days. Certain females also showed high indices, e.g. L13 (20·0) and L15 (22·4). A glance at these results shows conclusively that tremendous variation occurred in growth-rate and in efficiency in different individuals. As at Cawsand, the growth-rate was much slower than during 1929. Not a single fish rivalled the performances of L11, L15, and L16 of that year, which fish showed weight increases for a period of 154 days of 157·4, 140·7, and 175·0 gm. respectively (see Table 41 of my previous paper). This statement might also be applied to efficiency, with slight

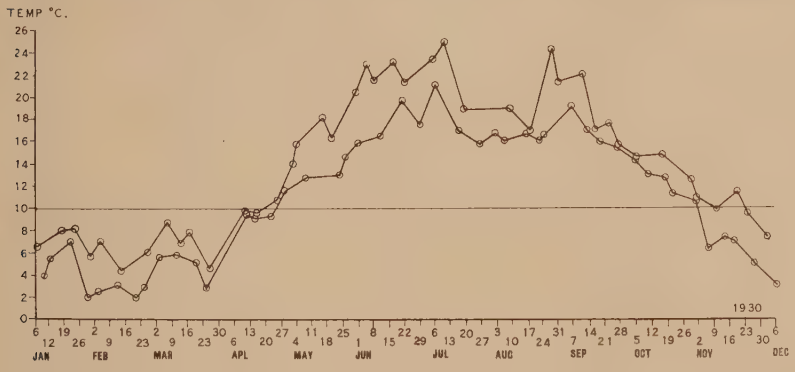

Fig. 9.—Graph of maximum and minimum temperatures at Lympstone, 1930.

reservation, since the fish mentioned showed indices of 7·1, 8·1, and 8·5. During 1930, L10b and L11b among males showed indices of 9·6 and 6·5 for periods of 98 and 42 days respectivly, L16a and b indices of 8·8 and 9·4 for periods of 42 and 126 days respectively, but no other fish showed such high efficiency as this over long periods (Table 58).

L10b showed a rapid growth-rate from one fortnightly period to another, with increments of 10·4, 7·7, 10·2, and 11·4 gm. and corresponding efficiency indices of 5·7, 8·7, 8·0, and 8·5 (Table 60). But it had not doubled its initial weight at the end of 112 days. L11b showed the remarkable fortnightly weight increases of 16·8 and 21·6 gm., with such high efficiency as indices of 4·4 and 4·8 indicate, yet this phenomenal growth-rate rapidly declined and during the next period only 2·3 gm. was added to the weight of the fish at a cost of 38·5 gm. of food per 1·0 gm. increase. These examples illustrate the slow rate of growth, variation in efficiency and irregularity of growth characterising the results of the Lympstone experiments with males. Other examples may be found readily in Tables 59–62. And these generalisations may well be applied to the

females also, where great variation occurs as characteristically as among males. The results for the females are shown in Tables 63–66, where details may be inspected readily. The growth-curves of weight for these females are presented in Figure 8.

Figure 9 shows graphically the maximum and minimum temperatures recorded for weekly periods from January 6th to December 6th, 1930, the data employed having been kindly sent to me by Mr. H. Lees.

4. Conclusions Drawn from the Experiments of 1930 (Lympstone).

(1) The results of the experiments of January to April indicate the fall in value of the maintenance requirements of both male and female plaice during the coldest months of the year. By comparing these results with those obtained for the same fishes during 1929, we see that in the case of a male fish approximately 50 gm. in weight (e.g. L1) the average ration required daily for maintenance falls from 0·9 gm. to 0·6 gm., i.e. by about one-third of its former value. The maintenance ratio diminishes from 0·017 to 0·011. In the case of a female approximately 100 gm. in weight, the daily ration falls from 1·3 gm. to 0·7 gm., the maintenance ratio from 0·013 to 0·007. The degree of reduction of these quantities varies considerably in different fish, but the results warrant the conclusion that during the colder months of the year, a plaice requires for purposes of maintenance only a fraction ($\frac{1}{2}$ to $\frac{2}{3}$) of the daily ration which is required to maintain the weight constant during warmer months.

(2) These early experiments also serve to show that maximum-fed plaice of one summer refuse even mere maintenance rations during the following winter months, whereas previously minimum-fed fish continue to take maintenance rations. Moreover, the former fish appear to require much smaller quantities of food for purposes of maintenance during the winter than do the latter fish.

(3) It is shown that at Lympstone, maximum feeding was completely resumed before the end of March and that it was attained in little more than a fortnight. Growth commenced at once and strikingly high efficiency was shown during the first few weeks.

(4) At Lympstone during the summer of 1930, maintenance requirements were in some cases much higher than at Cawsand, which might be expected in view of the fact that water temperatures were higher. The results of maintenance experiments are inconsistent in their variability, however, although they tend to support the Cawsand results in showing the order of the fall in value of the maintenance ratio with increase in size of fish.

(5) The growth-rate of maximum-feeding fish at Lympstone, as at Cawsand, was slow and the efficiency correspondingly low. These results similarly show the tremendous variation in rate of growth, and efficiency from one fortnightly period to another in the case of all fishes and from one fish to another at the same time. High efficiencies, e.g. 4·4 and 4·8 for L11b ; 4·5 and 6·8 for L13, were shown from time to time, but rarely. Lower efficiencies were common, and among both males and females for at least one half the total number of fortnightly periods, more than 15·0 gm. of food was required to produce 1·0 gm. increase in weight of the fish. Among males the efficiency index was less than 10·0 during only 10 periods out of a total of 52, among females during only 18 periods out of a total of 54, clearly very low degrees of efficiency.

III. GENERAL DISCUSSION OF RESULTS.

The experiments on maintenance in the plaice, which have extended over three years, have on the whole rendered fairly uniform and very conclusive results. We may lay claim to a considerable body of facts, which shows with some precision the order of the maintenance requirements of a two years' old plaice, not only at the commencement of, but also during and towards the end of the third season of growth. The results of these experiments directly apply to different fish of various sizes, but by a process of analogy, to which objection can scarcely be raised, they can be applied to the same fish at different times during the growth season with which we are concerned. Thus we arrive at an approximate evaluation of the maintenance requirements of fully-feeding, freely-growing plaice, using methods which are more in keeping with growth experiments than others which might have been selected, e.g. determination of maintenance requirements by study of respiratory exchanges. Data are available also to show how maintenance requirements diminish during the months when water temperatures are lowest, when plaice which have been fully-feeding have ceased to take food to any considerable extent and consequently have ceased to grow.

In a previous paper (Journ. Mar. Biol. Soc., Vol. XVII, No. 1, Sept. 1930, pp. 103–174) it was shown that the maintenance requirements of a 44 gm. male plaice are satisfied when an average daily ration of 0·6–0·7 gm. of Mytilus flesh is injested, but reasons were given for supposing that this ration value is low and that a more accurate value would be 0·7–0·8 gm., or 0·018 of the body weight (=maintenance ratio). During 1929, this statement was substantiated, for it was found that a 42 gm. male fish, C6a, maintained its weight constant over a period of 131 days when it took an average daily ration of 0·7 gm., which yields a maintenance ratio

of 0·017. It was shown further that C5a, a 30-gm. male, required a daily ration of 0·6 gm. for purposes of maintenance (ratio=0·019) and that the mean C fish, 17·6 gm. in weight, required 0·4 gm. per day (ratio=0·024). This was the closest approach that could be made to a determination of the increase in value of maintenance requirements with increase in size of the fish when the results of the Cawsand experiments of 1929 alone were employed. But it was seen that the increase in value of these requirements is not directly proportional to the increase in size, the maintenance ratio decreasing in value. By combining these results with the Lympstone results of the same year, it was possible to proceed a little way further. The male L1, a 52-gm. fish, required a daily ration of 0·9 gm. (maintenance ratio=0·017), while L2, a 130-gm. male, required 1·4 gm. per day (ratio=0·011). Indicating that objections might be raised to a combination of the two sets of results, the conclusion was nevertheless put forward that an increase in weight from 18 gm. to 130 gm. among male plaice entailed an increase in the daily ration required for maintenance from 0·4 gm. to 1·4 gm., which implies a decrease in the value of the maintenance ratio from 0·024 towards 0·011.

It is obviously both important and interesting to observe how far the results of 1930 conform to or depart from the conclusions ventured. Fortunately, at Cawsand during 1930, a far greater measure of success was met with in the experimental determination of maintenance requirements than had been met with during the previous two years, due to the arrangement of a graded series of individuals of various sizes representing stages passed through by a plaice during its third season of growth. And the results of this last year both strengthen and broaden the conclusions previously formed. In the text of this paper it has been shown that during 1930, the maintenance requirements of a male plaice 21·5 gm. in weight were approximately 0·46 gm. of Mytilus flesh daily, those of a similar fish 115·0 gm. in weight approximately 1·42 gm. of such food per day. The maintenance ratios corresponding to these quantities are 0·022 and 0·011. The close conformity between these results and those of the previous year is clearly seen and is also noteworthy.

It was suggested further, that the size difference indicated above is a close measure of the difference shown by a two-year-old plaice at the commencement of and towards the end of its third season of growth. We have thus reached the point at which it is possible to conclude, and with much faith in the accuracy of the conclusion, that about the commencement of the third season of growth a male plaice requires approximately 0·022 body-weight, or the first 0·4 gm. of its daily ration, to satisfy basal requirements and maintain its weight constant, and that towards the end of this season 0·011 body-weight, or the first 1·4 gm. of the daily ration for this purpose. The food is understood to be Mytilus flesh. The

variation in basal need with increase in size is presumably a steady one, so that it is possible to infer, with some experimental evidence to support the inference, that the maintenance ratio is a variable quantity which, during the season with which we are concerned, ranges from 0·022 towards 0·011. This is shown graphically from experimental results in Figure 1 (♂).

For females, corresponding success was met with during the final years' work, the results substantiating and amplifying the conclusions previously ventured. In my previous paper it is seen that at Cawsand during 1929 a female 22 gm. in weight required 0·4 gm. of food per day for maintenance, a similar fish 42 gm. in weight required 0·6 gm., or expressed in the form of maintenance ratios 0·019 and 0·015 of the body-weight respectively. A Lympstone female 103 gm. in weight required 1·3 gm. per day or 0·013 body-weight. The results of 1930 correspond more closely with those presented above for males. A female 17·3 gm. in weight required 0·43 gm. of food per day, a similar fish of 132·0 gm. required 1·24 gm. per day, the corresponding ratios being 0·022 and 0·009. The latter value appears to be subnormal in that the female A3, 101·5 gm. in weight, required 1·21 per day, yielding a maintenance ratio of 0·012, and it is distinctly possible that the maintenance requirements of females parallel those of males. It is unlikely that serious error would arise if the maintenance ratio of a female 130 gm. in weight was taken as being 0·011. In Figure 1 the results of these experiments are shown graphically for females as well as for males. These results can be extended in the way indicated above for fully-growing males.

The growth-rate shown at Cawsand during 1928 was rapid, the mean male increasing its initial weight of 38·8 gm. by 86·0 gm., the mean female increasing its initial weight of 43·3 gm. by 107·9 gm. during a period of 176 days. But the efficiency of these fish, as measured by the quantity of food required to produce an increase of 1·0 gm. in weight, was low, the mean male requiring 12·7 gm. and the mean female 10·5 gm. In the present paper it has been shown that during these preliminary experiments it is likely that there was a slight leakage of food materials through the coarse mesh of the box windows during the ebb and flow of tides. If this was the case, the efficiency indices shown are slightly greater than the true values. When the conditions and apparatus were refined, the efficiency shown was much higher, i.e. the indices were smaller. Thus during 1929 the average efficiency of D3, the most rapidly-growing male, for a period of 175 days was represented by the index 6·5, the average efficiencies of B1 and B4, the most rapidly-growing females, by the indices 7·6 and 7·2 respectively, over a corresponding period. Moreover, examination of the data for fortnightly periods showed that among Cawsand males, the efficiency was so high during certain periods as to yield an index of 4·8 and among the corresponding females an index of 5·2 was shown.

At Lympstone the efficiencies over long periods were lower, that of the male L11 being 7·1, those of the females L15, and L16 being 8·1 and 8·5 respectively. As at Cawsand, and to an even greater extent, higher efficiencies were shown during certain fortnightly periods, and generally there was much more variation in efficiency from period to period than at Cawsand.

At Cawsand during 1930, the highest efficiency over long periods was shown by the male D4, with an index of 9·1 for 116 days and the females B3 and B5, with indices of 9·7 and 8·5 for 165 and 87 days respectively. If these values are compared with those given for D3, B1, and B4 of 1929 above, it is seen that they are slightly greater, i.e. the efficiencies during 1930 were slightly lower. But the efficiencies shown during 1930 were higher than those shown during 1928, and we can safely infer, on the evidences of the results of two consecutive years, that the maximum average efficiency for periods approaching whole growth seasons in extent lies between efficiencies corresponding to the indices 6·5 and 9·1 for males, and 7·2 and 8·5 for females. In view of the fact that the higher efficiencies went side by side with more rapid, and perhaps more nearly typical, growth-rates, it would probably be safe to infer that they are more accurate from the viewpoint of normality.

Growth proceeded at a rapid rate at Cawsand during 1928, as is mentioned above. Such rapid rates and degrees of growth have not been shown since 1928, either at Cawsand or at Lympstone. This applies with additional force if we consider individual performances at Cawsand during the first year, when the male C3 increased its weight from 36·0 gm. to 158·0 gm., and the female D5 from 50·0 gm. to 203·0 gm., both performances taking 176 days. Such weight increase has not been equalled although the percentage growth has been exceeded. As has been shown, the above plaice increased their initial weights by over 300 per cent, while the later experiments yielded smaller actual weight increases but higher percentages of growth. Thus the Cawsand male D3 of 1929 increased in weight from 15·5 gm. to 105·0 gm., the corresponding females B1 and B4 from 24·0 gm. to 105·5 gm. and from 18·5 gm. to 103·5 gm. respectively, during 175 days, percentage increases of 580, 340, and 460 respectively. Actual weight increase is our chief concern however. It is obvious that any figures for percentage growth call for uniform initial weights and periods. The experiments of 1929 were started earlier than those of 1928, and with smaller fish. This fact causes percentage growth of 1929 to appear greater than that of 1928 despite the actually smaller weight increases. At Lympstone during 1929, slightly larger fish were used and, over a period of 154 days, the male L11 increased in weight from 23·2 gm. to 157·4 gm., the females L15 and L16 from 31·9 gm. to 140·7 gm. and from 50·5 gm. to 175·0 gm. respectively. Only L11 equalled

the performance of the Cawsand (1929) male C3 as regards actual weight increase.

For some reason which remains unknown, the rate of growth shown at both Cawsand and Lympstone during 1930 was much slower than that shown during 1929, degrees of growth being smaller also. The most actively-growing male, D1, increased in weight merely from 35·8 gm. to 77·1 gm. during 162 days, the most actively-growing female, B5, from 34·5 gm. to 82·5 gm. during 87 days. At Lympstone, L10b showed weight increase from 73·9 gm. to 127·7 gm. during 98 days, the female L16b, a three years' old fish presumably, from 104·9 gm. to 192·8 gm. during 126 days. These fishes were the most actively-growing individuals at Lympstone.

From the point of view of typical growth then, the results of 1930 are of much less value than those of the previous year. The experiments of 1929 are of most use in this respect, since they were commenced earlier in the year. But perhaps the best indication of maximum growth during the third season of the plaice is afforded when the results of 1929 are combined with those of 1928, i.e. when an hypothetical fish is built up of two individuals of consecutive years, taking care, of course, that the two periods do not overlap. Thus, if we take D3 (1928) and B4 (1929) as characteristic of females, it is seen that from May 22nd to July 19th, 1929, B4 increased its weight from 18·5 gm. to 43·0 gm., its length from 13·2 cm. to 16·1 cm. On July 16th, 1928, i.e. at the commencement of the experiment, D3 was 16·6 cm. in length and 43·0 gm. in weight. And it is reasonable to assume, bearing the performance of B4 in mind, that had the experiments of 1928 been commenced some 48 days earlier, they might have been commenced with a D3 fish of approximately 13 cm. in length and 18 gm. in weight, this being the size of B4 on May 22nd, 1929. Even when two sets of results are combined in this way, the whole growth season is not completely covered since, according to the Lympstone results of 1930, plaice are fully-feeding and freely-growing by the end of March, but a closer approximation is afforded for total growth during the third season.

By this means we can infer that the theoretical D3 fish of May 22nd, 1928, increased in size until by the end of December growth had taken place from 13 cm. and 18 gm. to 23·5 cm. and 155 gm. It might be considered objectionable to estimate growth to the end of December since during 1929 growth ceased before the end of November, but it should be realised that the degree of growth during December was slight. Moreover, as the duration of this theoretical growth season (221 days) has not been extended beyond the normal span (see below), no serious error is involved.

It is possible to repeat the above in the case of males, and by thus combining the results for C3 (1928) and D3 (1929) we can infer that growth

in weight from 15·5 gm. to 151 gm. takes place, theoretically, during the corresponding period. This approximation would hold as an index of maximum growth shown during the third season by a typical female. The method employed is open to objection, but the aim in view is an estimate of growth for the whole of the season with which we are concerned. Such methods will be discarded when it comes to attempting to estimate food requirements and food fractions going to satisfy maintenance requirements and to promote growth respectively. In attempting to do this the data of one particular year will be adhered to closely. Before leaving this question of total growth it might be mentioned that the Lympstone male, L11, increased in weight from 23·2 gm. to 147·2 gm. during 182 days ending December 23rd, 1929. This shows the estimates made above to be reasonable ones.

We might consider briefly Lympstone data which indicate the duration of the growth season. The results of the experiments of January to April show that the winter fast ceases quite abruptly during early March and that before the end of this month appetite is completely restored. About March 1st, 1930, the four Lympstone plaice L10–L15 were taking mere maintenance rations of approximately 2 gm. per day. On March 4th they commenced to take 4–5 gm. per day, and by March 6th were taking 5–6 gm. per day. The daily rations by the end of the month were 6–7 gm. It is clear that during the first week in March these plaice increased their daily rations from maintenance ones to almost maximum ones. We may consider that the feeding and growth season commences about the end of this week, say March 6th. At Lympstone during 1929, feeding ceased abruptly about November 11th, when maximum water temperatures approached 10°C., as the following data show. During the fortnight ending November 11th, L12, L13, and L15 took 50·0, 36·3, and 65·4 gm. of food respectively, while during the next fortnight only 8·4, 7·2, and 24·8 gm. respectively was taken. L15 continued partial feeding until December, but it is generally true that full feeding was discontinued about November 11th. And in each case this discontinuance marks the onset of a period when loss in weight occurs. The period of winter fasting thus extends over 16 weeks and includes the whole of December, January, and February. For about 36 weeks full feeding and unrestricted growth ensue. The growth period is seen to be about 30 days longer in extent than that corresponding to the total growth estimate provided above. Doubtless, some variation in duration of the growth period occurs, and at Lympstone during 1930, feeding and growth proceeded longer than during 1929, but after November 10th, when maximum water temperatures reached 10° C., feeding was partial, so that the above conclusions are not materially affected.

In my previous paper it was suggested that plaice which take food

rations intermediate in quantity between maintenance and maximum rations utilise food more efficiently for purposes of growth than do maximum-feeding plaice. It has been shown in the present paper that the evidence upon which this conclusion was based proved to be of doubtful accuracy. There is a possibility that slight quantities of food were washed out of the boxes during the early experiments and, if this actually occurred, the efficiency of the maximum-feeding fish was higher than the data indicate. The efficiency of these fish was used as a basis of comparison for intermediate-feeding fish and hence the higher efficiencies were possibly more apparent than real. But during 1930, this question was investigated under refined conditions, and more reliable data have been obtained.

Two fish of each sex were housed, in a segregated condition, in the modified fish boxes and were supplied with rations about twice the value of the maintenance rations. It is seen in Table 33 that these fish took an average daily ration of not more than 1·5 gm. over periods from 141–178 days, whereas the maximum-feeding fish took as much as 3·5 gm. per day. For the whole range of the experiments the I fish (intermediate-feeding) showed an unmistakably high degree of efficiency in the utilisation of food for growth purposes. No D or B fish (maximum-feeding) showed such low efficiency indices, i.e. high efficiency, as was shown by these fish for long periods, e.g. 6·8 (I1), 6·3 (I3), or 5·9 (I4). The indices for the two most efficient D fish are 9·1 (D4) and 10·2 (D3), those for the most efficient B fish, 8·5 (B5) and 9·7 (B3). Thus the male I1 required 2·3 gm. of food less than the male D4 did in order to increase its weight by 1·0 gm., the female I4 2·6 gm. less than the female B5 for corresponding weight increase. The conclusion that intermediate-feeding plaice are more efficient than maximum-feeding plaice seems perfectly justified. The evidences are presented in greater detail in the text of this paper.

Finally, it is intended to attempt to fulfil the principal aim of these experiments, which is to determine what portion of the maximum ration taken by a freely-growing plaice is used in satisfying basal requirements, i.e. supporting life by supplying the energy necessary for its maintenance, for tissue repair and replacement, and what portion remains for the promotion of growth. The results of the preliminary experiments of 1928 are of little or no use in this attempt since within 14 days of the commencement the maximum-feeding male fish were larger than the minimum-feeding fish. As the latter were retained for the whole course of the experiments it is not possible to compare the two sets fairly. In the case of females there was no fair basis of comparison from the very outset, on account of size differences existing then. In my previous paper an attempt was made to estimate the maintenance requirements of the fully-growing males, but it was based upon the assumption that the maintenance ratio

is constant throughout the third season of growth. Subsequent work has shown that such is not the case, the ratio decreasing with increase in size of the fish. But the estimate that approximately one-fifth of the total food ration was used in satisfying maintenance requirements compares as closely with the more refined estimate presented below as could be expected from the facts then available.

TABLE 67.

DATA CONCERNING THE MALE PLAICE D3 (1929).

Showing growth in weight, food taken, and variation in maintenance ratio with increasing size. Also food quantities estimated as having been used for purpose of maintenance, fraction of total food and efficiency as regards food available for growth.

Date (1929).	Weight. (gm.)	Food per period. (gm.)	Maintenance ratio (from Graph Fig. 1).	Days in period.	Estimated food required for maintenance per period. (gm.)	Fraction of total food used for maintenance.	Growth in weight per period. (gm.)	Efficiency (gm. of food required to produce 1·0 gm. increase in wt. of fish allowing for maintenance.)
June 6	15·5	—	—	—	—	—	—	—
,, 20	18·0	22·0	0·024	14	6·0	0·27	2·5	6·4
July 5	22·5	26·0	0·024	15	7·3	0·28	4·5	4·2
,, 19	30·0	36·0	0·020	14	7·4	0·21	7·5	3·8
Aug. 2	37·0	41·4	0·018	14	8·4	0·20	7·0	4·7
,, 16	41·5	44·2	0·0165	14	9·0	0·20	4·5	7·8
,, 30	51·5	53·2	0·015	14	9·8	0·18	10·0	4·3
Sept. 16	65·5	68·5	0·014	17	13·9	0·20	14·0	3·9
Oct. 1	79·0	65·2	0·0135	15	14·6	0·22	13·5	3·7
,, 15	90·0	76·5	0·013	14	15·4	0·20	11·0	5·6
,, 29	95·0	63·0	0·0125	14	16·2	0·26	5·0	9·4
Nov. 13	103·0	50·0	0·012	15	17·8	0·36	8·0	4·0
,, 28	105·0	39·0	0·012	15	18·7	0·48	2·0	10·1

During 1929 an attempt was made to remedy the defects of the early experiments by determining the maintenance requirements of fish of different sizes, but the attempt was not completely successful. It was intended to commence the experiments with minimum and maximum-feeding plaice of about the same size and to change the former periodically so as to be able to keep the sizes comparable throughout, but difficulty

was met with in the substitution of freshly-trawled fish for settled fish. The fish appear to require a period of time in which to settle down and segregation is not conducive to rapid settling down. However, the results of these experiments provided the valuable conclusions which have been mentioned above. Considerable advance was made during 1930 at

TABLE 68.

DATA CONCERNING THE FEMALE PLAICE B1 (1929).

Showing growth in weight, food taken, and variation in maintenance ratio with increasing size. Also food quantities estimated as having been used for purpose of maintenance, fraction of total food and efficiency as regards food available for growth.

Date (1929).	Weight. (gm.)	Food per period. (gm.)	Maintenance ratio (from Graph Fig. 1).	Days in period.	Estimated food required for maintenance per period. (gm.)	Fraction of total food used for maintenance.	Growth in weight per period. (gm.)	Efficiency (gm. of food required to produce 1·0 gm. increase in wt. of fish allowing for maintenance.)
May 22	24·0	—	—	—	—	—	—	—
June 6	31·0	40·0	0·019	15	7·8	0·20	7·0	4·6
,, 20	35·0	22·0	0·017	14	7·9	0·36	4·0	3·5
July 5	40·0	26·0	0·016	15	9·0	0·35	5·0	3·4
,, 19	47·0	38·0	0·015	14	9·1	0·24	7·0	4·1
Aug. 2	53·5	44·3	0·014	14	9·8	0·22	6·5	5·3
,, 16	61·0	46·2	0·0135	14	10·6	0·23	7·5	4·7
,, 30	69·0	57·0	0·0125	14	12·0	0·21	8·0	5·6
Sept. 16	79·5	69·0	0·012	17	15·1	0·22	10·5	5·1
Oct. 1	89·5	66·9	0·0115	15	14·6	0·22	10·0	5·2
,, 15	96·0	63·0	0·011	14	14·2	0·23	6·5	7·5
,, 29	98·5	49·0	0·011	14	15·0	0·31	2·5	13·6
Nov. 13	104·0	54·0	0·011	15	16·7	0·31	5·5	6·8
,, 27	105·5	43—	0·011	14	16·1	0·37+	1·5	18·0+

Cawsand, what is perhaps the best method of experimenting being employed, the arrangement at the outset of a graded series of fish such as has been described previously. It remains only to apply the results obtained to the estimation it is intended to make. By the use of the graphs shown in Figure 1 it is possible to read off the maintenance ratios of steadily-growing plaice from one fortnightly period to another, using the

mid-body weights for the periods concerned, and thus estimate approximately the quantity of food required for maintenance, which can then be expressed as a fraction of the total quantity of food taken.

Probably, the most useful estimate will be provided if the performances of plaice showing typical sigmoid growth-curves are made use of. The

TABLE 69.

DATA CONCERNING THE FEMALE PLAICE B4 (1929).

Showing growth in weight, food taken, and variation in maintenance ratio with increasing size. Also food quantities estimated as having been used for purpose of maintenance, fraction of total food and efficiency as regards food available for growth.

Date (1929).	Weight. (gm.)	Food per period. (gm.)	Maintenance ratio (from Graph Fig. 1).	Days in period.	Estimated food required for maintenance per period. (gm.)	Fraction of total food used for maintenance.	Growth in weight per period. (gm.)	Efficiency (gm. of food required to produce 1·0 gm. increase in wt. of fish allowing for maintenance.)
May 22	18·5	—	—	—	—	—	—	—
June 6	26·0	40·0	0·0215	15	7·2	0·18	7·5	4·4
,, 20	32·0	22·0	0·0185	14	7·5	0·34	6·0	2·4
July 5	36·5	26·0	0·017	15	8·7	0·33	4·5	3·8
,, 19	43·0	36·5	0·016	14	8·9	0·24	6·5	4·2
Aug. 2	50·5	48·7	0·015	14	9·8	0·20	7·5	5·2
,, 16	60·5	51·4	0·0135	14	10·5	0·20	10·0	4·1
,, 30	66·0	54·3	0·013	14	10·9	0·20	5·5	7·9
Sept. 16	76·0	65·5	0·0125	17	15·1	0·23	10·0	5·0
Oct. 1	83·5	55·2	0·012	15	14·4	0·26	7·5	5·3
,, 15	90·0	59·0	0·0115	14	14·0	0·24	6·5	6·9
,, 29	102·0	63·0	0·011	14	14·8	0·23	12·0	4·0
Nov. 13	108·0	52·5	0·011	15	17·2	0·34	6·0	5·9
,, 27	103·5	38·5	0·011	14	16·3	0·42	4·5	—

growth performances during 1930 are apparently subnormal, those of 1928 are marred by the possibility of food leakages. Undoubtedly the results obtained in the experiments with the male D3 and the females B1 and B4 are likely to be of most use in this respect. Accordingly these will be employed.

In Table 67, which is partly derived from data shown in Table 20 of

my previous paper, are shown the weights of D3 on a succession of weighing days from June 6th to November 28th, 1929. The maintenance ratios, obtained from the graph in Figure 1, are shown in col. 4, while in col. 6 is shown the estimated maintenance requirements for the periods, these being given as fractions of the total food in col. 7. It is seen that for the period ending June 20th, 6·0 gm., or 0·27 of the total food taken, was required for maintenance and that for the period ending October 29th, 16·2 gm., or 0·26 of the total, was required. From June 6th to October

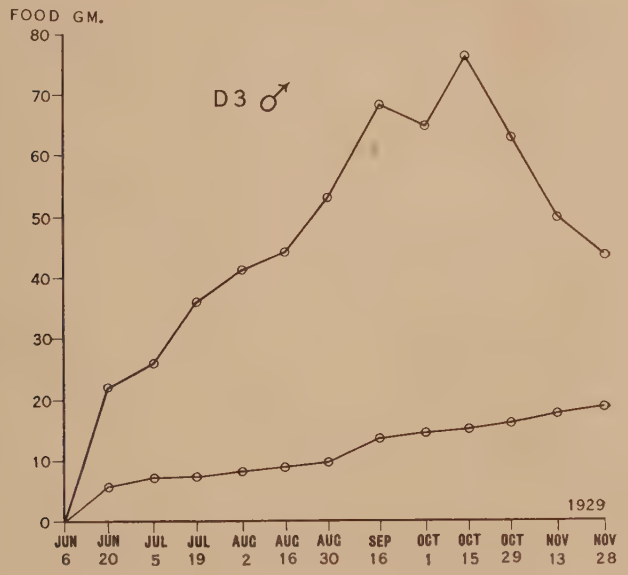

FIG. 10.—Acceleration curves showing the relationship between the total quantity of food taken by D3 (♂) during 1929 and the quantities estimated as having been used for purposes of maintenance. Above, total food; below, food for maintenance.

29th the fraction for the various fortnightly periods varied between 0·18 and 0·28. These results are shown graphically in Figure 10. During November, when feeding was becoming inhibited, the fraction was increased, but the figures quoted do not take into consideration the slight fall in value of the maintenance ratio which would undoubtedly occur here. For the whole of the experiment, 177 days, the total food taken was 585 gm. of which, according to this estimate, 145 gm. have been required to satisfy basal requirements, i.e. approximately 25 per cent of the total. In the last column of Table 67 are shown indices of efficiency relating to food available for the promotion of growth, and it is seen that these vary

from period to period up till the end of October between 3·7 and 9·4. Thus, no matter whether efficiency is estimated in terms of total food or in terms of food available for growth, the efficiency index is not constant for successive short periods during the growth season. But the table indicates that the index is, in round numbers, 4 during each of six periods and greater than 4 during six others. It appears that of food available for growth purposes every 4 gm. generally produces 1·0 gm. of fish.

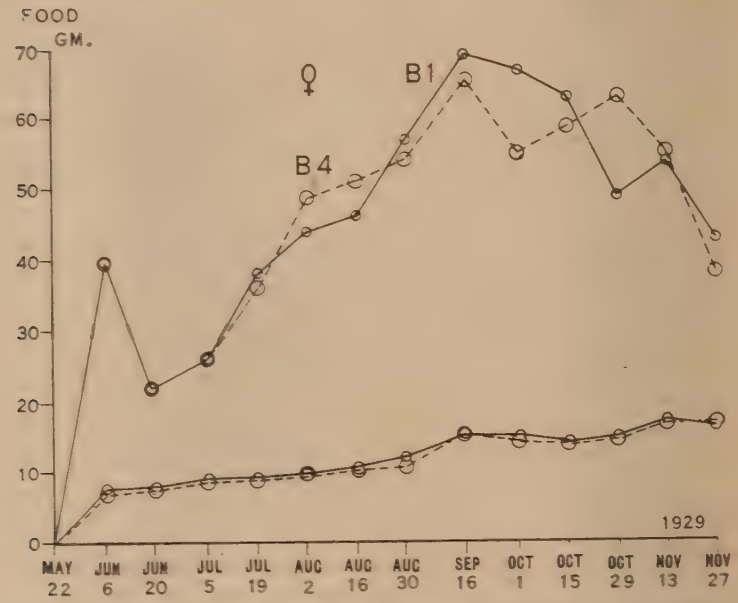

FIG. 11.—Acceleration curves showing the relationship between the total quantities of food taken by B1 and B4 (♀) during 1929 and the quantities estimated as having been used for purposes of maintenance. Above, total food: below, food for maintenance.

Tables 68 and 69 present data for the females B1 and B4, the method of obtaining them being precisely the same as that used for D3. For the period of 15 days ending June 6th, B1 required 7·8 gm. of food for maintenance, 0·20 of the total quantity taken, while for the period of 14 days ending October 29th it required 15·0 gm. or 0·31 of the total quantity taken (Table 68). For the whole experiment, 189 days, 618 gm. of food was taken, of which 158 gm. is estimated as having been required for maintenance, i.e. approximately 25 per cent of the total. These results are shown graphically in Figure 11. In the case of B4 7·2 gm. of food or 0·18 of the total quantity taken was required for maintenance during 15 days to June 6th, 14·8 gm. or 0·23 of the total during 14 days to October

29th. During 187 days a total of 613 gm. of food was taken, of which 155 gm. was used for purposes of maintenance, again approximately 25 per cent of the total (Table 69). It is seen that the efficiency as regards food available for growth was just as inconstant as in the cases of B1 and D3. But for five periods the efficiency index was, in round numbers, 4, and for three periods 5, from which it can be inferred that generally between 4 and 5 gm. of food available for growth produce 1·0 gm. of fish. For B1 it appears that 5 gm. was required to produce this weight of fish, the efficiency shown being slightly less than that shown by B4. For the whole experiment the average efficiencies read as follows : B1—5·4, B4—4·9, and D3—4·8, the male thus showing the greatest degree of efficiency.

The general conclusions afforded by these estimates are uniform for males and females alike. Of the total food taken during the growth season, approximately 25 per cent is used for maintenance. Of the remainder which is available for the promotion of growth, on the average for the season and expressed in round numbers each 5 gm. produces 1·0 gm. increase in weight of the fish, although for shorter periods 4 gm., or even slightly less, may produce such an increase.

The discussion part of this paper must serve as a summary of results, since to summarise further would scarcely serve a useful purpose. In many respects it is considered that the treatment of the available data by the methods adopted is scarcely adequate, and it is hoped to be able to treat these data by strictly statistical methods in the near future. When the methods of correlation are applied to the data, it is likely that reliable generalised results will be forthcoming which might possibly have a greater practical application in fishery research.

IV. Acknowledgement.

I wish to record my cordial thanks to Dr. E. J. Allen who in very many ways has helped to meet the difficulties that have arisen during the course of this work and who by consistent kindness has caused very tedious work to seem much less tedious, for which I am very grateful. Dr. E. S. Russell and Mr. T. Edser have been ever ready to help with suggestions and criticism and to them also is recorded a mark of my appreciation. Thanks are also due to Mr. H. Lees for the care he has shown in his charge of the Lympstone experiments under the direction of Dr. Russell and Mr. Edser, and to all members of the staff of the Marine Biological Association who have assisted in the work. Mr. Seale and the captain and crew of the *Salpa* have also rendered valuable service and to them also my thanks are tendered.

TABLES 1–6.

Data Obtained from the Experiments on the Maintenance Requirements of Male Plaice.

Table 1: C1.

Date (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 14	13·1	21·5	—	—	—	—	—	—	—	—	—	—
,, 26	13·0	20·5	− 0·1	− 1·0	4·2	12	0·35	0·017	4·5	12	− 0·1	− 1·0
June 9	13·0	19·3	nil	− 1·2	3·4	14	0·24	0·012	7·9	26	− 0·1	− 2·2
,, 23	13·0	17·8	nil	− 1·5	3·4	14	0·24	0·013	11·3	40	− 0·1	− 3·7
July 7	13·0	19·3	nil	1·5	8·5	14	0·61	0·031	19·8	54	− 0·1	− 2·2
,, 21	13·1	20·0	0·1	0·7	7·1	14	0·51	0·026	26·9	68	nil	− 1·5
Aug. 6	13·3	21·0	0·2	1·0	8·0	16	0·50	0·024	34·9	84	0·2	− 0·5
,, 21	13·3	21·5	nil	0·5	7·5	15	0·50	0·024	42·4	99	0·2	nil
Sept. 5	13·3	20·7	nil	− 0·8	6·0	15	0·40	0·019	48·4	114	0·2	− 0·8
,, 19	13·3	20·7	nil	nil	8·5	14	0·61	0·030	56·9	128	0·2	− 0·8
Oct. 5	13·4	20·8	0·1	0·1	10·0	16	0·62	0·030	66·9	144	0·3	− 0·7

Table 2: C2.

May 14	15·3	31·5	—	—	—	—	—	—	—	—	—	—
,, 26	15·3	31·6	nil	0·1	7·8	12	0·65	0·021	7·8	12	nil	0·1
June 9	15·4	32·5	0·1	0·9	6·9	14	0·49	0·015	14·7	26	0·1	1·0
,, 23	15·5	32·3	0·1	− 0·2	6·4	14	0·46	0·014	21·1	40	0·2	0·8
July 7	15·6	33·2	0·1	0·9	7·0	14	0·50	0·015	28·1	54	0·3	1·7
,, 21	15·8	34·0	0·2	0·8	6·6	14	0·47	0·014	34·7	68	0·5	2·5
Aug. 6	15·9	35·0	0·1	1·0	7·5	16	0·47	0·014	42·2	84	0·6	3·5
,, 21	15·9	35·2	nil	0·2	7·4	15	0·49	0·014	49·6	99	0·6	3·7
Sept. 5	16·0	35·0	0·1	−0·2	7·0	15	0·47	0·013	56·6	114	0·7	3·5
,, 19	16·0	35·4	nil	0·4	7·0	14	0·45	0·013	63·6	128	0·7	3·9
Oct. 6	16·1	36·5	0·1	1·1	8·5	17	0·50	0·014	72·1	145	0·8	5·0
,, 22	16·1	36·8	nil	0·3	5·8	16	0·41	0·011	77·9	161	0·8	5·3
Nov. 6	16·2	35·9	0·1	− 0·9	6·0	15	0·40	0·011	83·9	176	0·9	4·4

Table 3: C3.

May 28	16·9	45·0	—	—	—	—	—	—	—	—	—	—
June 9	17·0	46·5	0·1	1·5	9·3	12	0·77	0·016	9·3	12	0·1	1·5
,, 23	17·2	46·5	0·2	nil	8·0	14	0·57	0·012	17·3	26	0·3	1·5
July 7	17·3	48·4	0·1	1·9	8·0	14	0·57	0·012	25·3	40	0·4	3·4
,, 21	17·4	48·5	0·1	0·1	7·6	14	0·54	0·011	32·9	54	0·5	3·5
Aug. 6	17·4	46·0	nil	− 2·5	8·2	16	0·51	0·011	41·1	70	0·5	1·0
,, 21	17·3	44·8	− 0·1	− 1·2	8·0	15	0·53	0·012	49·1	85	0·4	− 0·2
Sept. 5	17·2	42·9	− 0·1	− 1·9	8·0	15	0·53	0·012	57·1	100	0·3	− 2·1
,, 19	17·4	42·7	0·2	− 0·2	11·5	14	0·82	0·019	68·6	114	0·5	− 2·3
Oct. 6	17·4	44·1	nil	1·4	14·0	17	0·81	0·019	82·6	131	0·5	− 0·9
,, 22	17·4	44·1	nil	nil	10·8	16	0·67	0·015	93·4	147	0·5	− 0·9
Nov. 6	17·4	43·0	nil	− 1·1	10·3	15	0·70	0·016	103·7	162	0·5	− 2·0

Table 4 : C4.

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt. (gm.)
May 12	18·9	59·0	—	—	—	—	—	—	—	—	—	—
,, 26	18·9	61·0	nil	2·0	11·3	14	0·81	0·013	11·3	14	nil	2·0
June 9	19·0	59·6	0·1	− 1·4	11·9	14	0·85	0·014	23·2	28	0·1	0·6
,, 23	19·0	61·0	nil	1·4	14·0	14	1·00	0·017	37·2	42	0·1	2·0
July 7	19·0	61·2	nil	0·2	13·0	14	0·93	0·015	50·2	56	0·1	2·2
,, 21	19·1	63·0	0·1	1·8	12·1	14	0·86	0·014	62·3	70	0·2	4·0
Aug. 6	19·2	62·2	0·1	− 0·8	12·4	16	0·77	0·012	74·7	86	0·3	3·2
,, 21	19·2	61·0	nil	− 1·2	12·5	15	0·83	0·013	87·2	101	0·3	2·0
Sept. 5	19·2	60·1	nil	− 0·9	12·0	15	0·80	0·013	99·2	116	0·3	1·1
,, 19	19·2	61·8	nil	1·7	17·5	14	1·25	0·020	116·7	130	0·3	2·8
Oct. 6	19·3	62·8	0·1	1·0	18·5	17	1·09	0·016	135·2	147	0·4	3·8
,, 22	19·4	62·8	0·1	nil	15·0	16	0·94	0·015	150·2	163	0·5	3·8
Nov. 6	19·3	62·5	− 0·1	− 0·3	7·5	15	0·50	0·008	157·7	178	0·4	3·5

Table 5 : C5.

May 26	21·2	86·7	—	—	—	—	—	—	—	—	—	—
June 9	21·3	86·5	0·1	− 0·2	13·8	14	0·99	0·011	13·8	14	0·1	− 0·2
,, 23	21·4	87·5	0·1	1·0	13·8	14	0·99	0·011	27·6	28	0·2	0·8
July 7	21·4	88·2	nil	0·7	13·0	14	0·93	0·011	40·6	42	0·2	1·5
,, 21	21·4	89·0	nil	0·8	12·3	14	0·88	0·010	52·9	56	0·2	2·3
Aug. 6	21·4	86·5	nil	− 2·5	13·4	16	0·84	0·010	66·3	72	0·2	− 0·2
,, 21	21·4	87·2	nil	0·7	13·5	15	0·90	0·010	79·8	87	0·2	0·5
Sept. 5	21·5	85·2	0·1	− 2·0	13·0	15	0·87	0·010	92·8	102	0·3	− 1·5
,, 19	21·4	84·3	− 0·1	− 0·9	15·5	14	1·11	0·013	108·3	116	0·2	− 2·4
Oct. 6	21·5	85·6	0·1	1·3	19·0	17	1·12	0·013	127·3	133	0·3	− 1·1
,, 22	21·5	85·6	nil	nil	16·0	16	1·00	0·012	143·3	149	0·3	− 1·1
Nov. 6	21·5	85·4	nil	− 0·2	13·0	15	0·87	0·010	156·3	164	0·3	− 1·3

Table 6 : C6.

June 12	23·0	115·0	—	—	—	—	—	—	—	—	—	—
,, 23	23·0	113·0	nil	− 2·0	14·7	11	1·34	0·012	14·7	11	nil	− 2·0
July 7	23·0	112·8	nil	− 0·2	19·5	14	1·39	0·012	34·2	25	nil	− 2·2
,, 21	22·8	115·0	− 0·2	2·2	19·8	14	1·41	0·012	54·0	39	− 0·2	nil
Aug. 6	22·7	113·2	− 0·1	− 1·8	19·8	16	1·24	0·011	73·8	55	− 0·3	− 1·8
,, 21	22·8	113·2	0·1	nil	19·0	15	1·27	0·011	92·8	70	− 0·2	− 1·8
Sept. 5	22·7	110·0	− 0·1	− 3·2	18·5	15	1·23	0·011	111·3	85	− 0·3	− 5·0
,, 19	22·8	109·5	0·1	− 0·5	25·0	14	1·79	0·016	136·3	99	− 0·2	− 5·5
Oct. 6	22·7	109·8	− 0·1	0·3	27·7	17	1·63	0·015	164·0	116	− 0·3	− 5·2
,, 22	22·8	112·5	0·1	2·7	25·2	16	1·57	0·014	189·2	132	− 0·2	− 2·5
Nov. 6	22·8	111·1	nil	− 1·4	19·3	15	1·29	0·012	208·5	147	− 0·2	− 3·9

TABLE 7.

SUMMARISED DATA CONCERNING THE MAINTENANCE REQUIREMENTS OF THE MALE PLAICE, C1–C6.

	Initial size.		Final size.		Growth.				
Fish No.	Lth. cm.	Wt. gm.	Lth. cm.	Wt. gm.	Lth. cm.	Wt. gm.	Days in period.	Average weight of food per day. (gm.)	Ratio Food per day/ /mid body-weight per period.
C1	13·1	21·5	13·4	20·8	0·3	−0·7	144	0·46	0·022
C2	15·3	31·5	16·2	35·9	0·9	4·4	176	0·49	0·015
C3	16·9	45·0	17·4	43·0	0·5	−2·0	162	0·64	0·015
C4	18·9	59·0	19·3	62·5	0·4	3·5	178	0·89	0·015
C5	21·2	86·7	21·5	85·4	0·3	−1·3	164	0·95	0·011
C6	23·0	115·0	22·8	111·1	−0·2	−3·9	147	1·42	0·013

TABLES 8–13.

Data Obtained from the Experiments on the Maintenance Requirements of Female Plaice.

Table 8: A5.

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt. (gm.)
June 16	12·2	17·3	—	—	—	—	—	—	—	—	—	—
,, 23	12·4	17·8	0·2	0·5	3·5	7	0·50	0·029	3·5	7	0·2	0·5
July 7	12·6	19·0	0·2	1·2	8·0	14	0·57	0·031	11·5	21	0·4	1·7
,, 21	12·8	20·0	0·2	1·0	6·6	14	0·47	0·025	18·1	35	0·6	2·7
Aug. 6	12·9	20·5	0·1	0·5	7·5	16	0·47	0·023	25·6	51	0·7	3·2
,, 21	13·0	21·2	0·1	0·7	6·6	15	0·44	0·021	32·2	66	0·8	3·9
Sept. 5	13·2	20·6	0·2	−0·6	6·0	15	0·40	0·019	38·2	81	1·0	3·3
,, 19	13·2	21·8	nil	1·2	5·5	14	0·39	0·018	43·7	95	1·0	4·5
Oct. 6	13·3	22·3	0·1	0·5	8·0	17	0·47	0·021	51·7	112	1·1	5·0
,, 22	13·3	22·3	nil	nil	5·5	16	0·34	0·015	57·2	128	1·1	5·0
Nov. 6	13·3	22·0	nil	−0·3	5·0	15	0·33	0·015	62·2	143	1·1	4·7

Table 9: A6 (a and b).

May 14	15·6	33·0	—	—	—	—	—	—	—	—	—	—
,, 26	15·5	31·8	−0·1	−1·2	6·3	12	0·52	0·016	6·3	12	−0·1	−1·2
June 9	15·5	30·8	nil	−1·0	6·3	14	0·45	0·014	12·6	26	−0·1	−2·2
,, 23	15·5	30·1	nil	−0·7	3·9	14	0·28	0·009	16·5	40	−0·1	−2·9
July 7	15·5	29·1	nil	−1·0	6·2	14	0·44	0·015	22·7	54	−0·1	−3·9
,, 21	15·3	28·0	−0·2	−1·1	1·0	14	0·04	—	23·7	68	−0·3	−5·0
Aug. 6	15·3	27·0	nil	−1·0	5·2	16	0·32	0·012	28·9	84	−0·3	−6·0
Aug. 22	15·2	35·0	—	—	—	—	—	-	—	—	—	—
Sept. 5	15·2	34·2	nil	−0·8	3·5	15	0·24	0·007	3·5	15	nil	−0·8
,, 19	15·2	32·3	nil	−1·9	4·6	14	0·33	0·010	8·1	29	nil	−2·7
Oct. 6	15·2	31·5	nil	−0·8	5·6	17	0·33	0·010	13·7	46	nil	−3·5
,, 22	15·2	30·2	nil	−1·3	4·9	16	0·31	0·010	18·6	62	nil	−4·8
Nov. 6	15·2	30·1	nil	−0·1	7·6	15	0·51	0·017	26·2	77	nil	−4·9

Table 10: A2.

June 11	17·3	49·0	—	—	—	—	—	—	—	—	—	—
,, 23	17·3	46·8	nil	−2·2	9·0	12	0·75	0·016	9·0	12	nil	−2·2
July 7	17·5	50·8	0·2	4·0	11·0	14	0·79	0·016	20·0	26	0·2	1·8
,, 21	17·5	52·0	nil	1·2	9·0	14	0·64	0·012	29·0	40	0·2	3·0
Aug. 6	17·6	51·0	0·1	−1·0	9·5	16	0·59	0·011	38·5	56	0·3	2·0
,, 21	17·6	52·0	nil	1·0	10·0	15	0·67	0·013	48·5	71	0·3	3·0
Sept. 5	17·6	49·4	nil	−2·6	10·0	15	0·67	0·013	58·5	86	0·3	0·4
,, 19	17·6	52·2	nil	2·8	11·5	14	0·82	0·016	70·0	100	0·3	3·2
Oct. 6	17·6	53·5	nil	1·3	14·0	17	0·82	0·016	84·0	117	0·3	4·5
,, 22	17·7	53·1	0·1	−0·4	11·0	16	0·69	0·013	95·0	133	0·4	4·1
Nov. 6	17·7	53·0	nil	−0·1	7·0	15	0·47	0·009	102·0	148	0·4	4·0

Table 11: A4.

Date (1930)	Size of fish. Lth. (cm.)	Wt. (gm.)	Growth per period. Lth. (cm.)	Wt. (gm.)	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/average body-wt. for period	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth. Lth. (cm.)	Wt. (gm.)
May 12	19·8	71·0	—	—	—	—	—	—	—	—	—	—
,, 26	19·8	67·7	nil	− 3·3	12·0	14	0·86	0·012	12·0	14	nil	− 3·3
June 9	19·9	67·9	0·1	0·2	11·7	14	0·84	0·012	23·7	28	0·1	− 3·1
,, 23	20·0	67·8	0·1	− 0·1	11·7	14	0·84	0·012	35·4	42	0·2	− 3·2
July 7	20·1	67·7	0·1	− 0·1	13·0	14	0·93	0·014	48 4	56	0·3	− 3·3
,, 21	20·1	68·0	nil	0·3	13·0	14	0·93	0·014	61·4	70	0·3	− 3·0
Aug. 6	20·2	68·5	0·1	0·5	14·5	16	0·91	0·013	75·9	86	0·4	− 2·5
,, 21	20·2	66·5	nil	− 2·0	13·0	15	0·87	0·013	88·9	101	0·4	− 4·0
Sept. 5	20·4	66·3	0·2	− 0·2	13·5	15	0·90	0·014	102·4	116	0·6	− 4·7
,, 19	20·2	66·4	− 0·2	0·1	13·5	14	0·96	0·014	115·9	130	0·4	− 4·6
Oct. 6	20·3	68·6	0·1	2·2	16·3	17	0·96	0·014	132·2	147	0·5	− 2·4
,, 22	20·4	68·8	0·1	0·2	14·5	16	0·91	0·013	146·7	163	0·6	− 2·2
Nov. 6	20·2	68·3	− 0·2	− 0·5	8·3	15	0·55	0·008	155·0	178	0·4	− 2·7

Table 12: A3.

June 12	22·0	101·5	—	—	—	—	—	—	—	—	—	—
,, 23	22·0	97·5	nil	− 4·0	13·0	11	1·18	0·012	13·0	11	nil	− 4·0
July 7	22·0	97·9	nil	0·4	17·5	14	1·25	0·013	30·5	25	nil	− 3·6
,, 21	21·9	100·0	− 0·1	2·1	17·0	14	1·21	0·012	47·6	39	− 0·1	− 1·5
Aug. 6	22·0	97·0	0·1	− 3·0	18·5	16	1·16	0·012	66·0	55	nil	− 4·5
,, 21	22·0	96·0	nil	− 1·0	17·5	15	1·17	0·012	83·5	70	nil	− 5·5
Sept. 5	22·2	93·5	0·2	− 2·5	17·0	15	1·13	0·012	100·5	85	0·2	− 8·0
,, 19	21·8	95·6	− 0·4	2·1	20·0	14	1·43	0·015	120·5	99	− 0·2	− 5·9
Oct. 6	21·9	96·8	0·1	1·2	24·5	17	1·44	0·015	145·0	116	− 0·1	− 4·7
,, 22	22·0	96·3	0·1	− 0·5	20·6	16	1·29	0·013	165·6	132	nil	− 5·2
Nov. 6	22·0	95·2	nil	− 1·1	13·0	15	0·87	0·009	178·6	147	nil	− 6·3

Table 13: A1.

May 12	24·5	132·0	—	—	—	—	—	—	—	—	—	—
,, 26	24·6	133·8	0·1	1·8	13·7	14	0·98	0·007	13·7	14	0·1	1·8
June 9	24·6	132·0	nil	− 1·8	19·0	14	1·36	0·010	32·7	28	0·1	nil
,, 23	24·6	134·0	nil	2·0	21·0	14	1·50	0·011	53·7	42	0·1	2·0
July 7	24·6	133·2	nil	− 0·8	18·5	14	1·32	0·010	72·2	56	0·1	1·2
,, 21	24·6	138·0	nil	4·8	18·5	14	1·32	0·010	90·7	70	0·1	6·0
Aug. 6	24·8	136·0	0·2	− 2·0	20·0	16	1·25	0·009	110·7	86	0·3	4·0
,, 21	24·7	136·0	− 0·1	nil	19·0	15	1·27	0·009	129·7	101	0·2	4·0
Sept. 5	24·7	136·2	nil	0·2	18·5	15	1·23	0·009	148·2	116	0·2	4·2
,, 19	24·7	135·5	nil	− 0·7	18·5	14	1·32	0·010	166·7	130	0·2	3·5
Oct. 6	24·7	140·4	nil	4·9	21·5	17	1·26	0·009	188·2	147	0·2	8·4
,, 22	24·8	140·8	0·1	0·4	20·0	16	1·25	0·009	208·2	163	0·3	8·8
Nov. 6	24·7	136·9	− 0·1	− 3·9	13·0	15	0·87	0·006	221·2	178	0·2	4·9

TABLE 14.

SUMMARISED DATA CONCERNING THE MAINTENANCE REQUIREMENTS OF THE FEMALE PLAICE, A1–A6.

Fish No.	Initial size. Lth. (cm.)	Initial size. Wt. (gm.)	Final size. Lth. (cm.)	Final size. Wt. (gm.)	Growth. Lth. (cm.)	Growth. Wt. (gm.)	Days in period.	Average weight of food per day. (gm.)	Ratio Food per day/ /mid body-weight per period.
A5	12·2	17·3	13·3	22·0	1·1	4·7	143	0·43	0·022
A6a	15·6	33·0	15·3	27·0	—0·3	—6·0	84	0·34	0·011
b	15·2	35·0	15·2	30·1	nil	—4·9	77	0·34	0·010
A2	17·3	49·0	17·7	53·0	0·4	4·0	148	0·69	0·014
A4	19·8	71·0	20·2	68·3	0·4	—2·7	178	0·87	0·012
A3	22·0	101·5	22·0	95·2	nil	—6·3	147	1·21	0·012
A1	24·5	132·0	24·7	136·9	0·2	4·9	178	1·24	0·009

TABLE 15.

SUMMARISED DATA CONCERNING MAXIMUM REQUIREMENTS AND GROWTH IN MALE AND FEMALE PLAICE.

Fish No.	Sex.	Initial size.		Final size.		Growth.		Period.		Days in Period.	Total food per the period. (gm.)	Average weight of food per day for the period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.
		Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	From	To				
D1	♂	15·0	35·8	19·2	77·1	4·2	41·3	28 : 5	6 : 11	162	475	3·0	11·5
D2	♂	13·5	22·0	17·4	52·1	3·9	30·1	12 : 5	6 : 11	178	358	2·0	11·9
D3	♂	14·0	26·5	17·4	51·0	3·4	24·5	12 : 5	5 : 9	116	250	2·2	10·2
D4	♂	13·5	23·0	16·4	44·5	2·9	21·5	12 : 5	5 : 9	116	196	1·7	9·1
D5	♂	14·6	31·7	16·8	47·2	2·2	15·5	9 : 6	5 : 9	88	159	1·8	10·3
D6	♂	13·1	18·2	15·1	33·0	2·0	14·8	29 : 5	5 : 9	99	159	1·6	10·7
B1	♀	14·7	26·7	18·0	55·7	3·3	29·0	17 : 6	6 : 11	142	331	2·3	11·4
B2	♀	15·5	30·5	18·2	64·1	2·7	33·6	17 : 6	6 : 11	142	352	2·5	10·5
B3	♀	12·9	19·7	17·3	50·3	4·4	30·6	26 : 5	6 : 11	165	296	1·8	9·7
B5	♀	15·5	34·5	19·2	82·5	3·7	48·0	26 : 5	21 : 8	87	308	3·5	8·5
B4	♀	12·3	17·2	16·6	39·1	4·3	21·9	26 : 5	6 : 10	134	256	1·9	11·7
B6	♀	14·3	27·5	16·4	45·6	2·1	18·1	12 : 5	20 : 9	131	233	1·8	12·9

TABLES 16–21.

Maximum Requirements and Growth of Male Plaice.

Table 16 : D1.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 28	15·0	35·8	0·011	—	—	—	—	—	—	—	—	—	—
June 9	16·2	46·0	0·011	1·2	10·2	43·3	4·2	12	3·61	28·5	43	1·2	10·2
,, 23	17·0	51·3	0·010	0·8	5·3	57·1	10·8	14	4·08	11·5	100	2·0	15·5
July 7	17·3	52·5	0·010	0·3	1·2	35·3	29·4	14	2·52	2·3	136	2·3	16·7
,, 21	17·6	58·0	0·011	0·3	5·5	40·0	7·3	14	2·86	10·5	176	2·6	22·2
Aug. 6	17·8	57·7	0·010	0·2	−0·3	38·6	—	16	2·41	—	214	2·8	21·9
,, 21	18·1	60·5	0·010	0·3	2·8	32·2	11·5	15	2·15	5·0	246	3·1	24·7
Sept. 5	18·3	60·8	0·010	0·2	0·3	41·1	137·0	15	2·74	0·5	288	3·3	25·0
,, 20	18·4	64·3	0·010	0·1	3·5	39·7	11·3	15	2·65	5·8	327	3·4	28·5
Oct. 6	18·8	70·0	0·011	0·4	5·7	54·1	9·5	16	3·38	8·9	381	3·8	34·2
,, 22	19·1	72·2	0·011	0·3	2·2	44·8	20·4	16	2·80	3·1	426	4·1	36·4
Nov. 6	19·2	77·1	0·011	0·1	4·9	48·5	9·9	15	3·23	6·8	475	4·2	41·3

Table 17 : D2.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	13·5	22·0	0·009	—	—	—	—	—	—	—	—	—	—
,, 26	13·5	22·7	0·009	nil	0·7	14·0	20·0	14	1·00	3·2	14	nil	0·7
June 9	14·3	29·7	0·010	0·8	7·0	30·2	4·3	14	2·16	30·8	44	0·8	7·7
,, 23	15·0	33·0	0·010	0·7	3·3	36·0	10·9	14	2·58	11·1	80	1·5	11·0
July 7	15·3	35·2	0·010	0·3	2·2	26·8	12·2	14	1·91	6·7	107	1·8	13·2
,, 21	15·7	39·0	0·010	0·4	3·8	32·7	8·6	14	2·34	10·8	140	2·2	17·0
Aug. 6	16·1	43·2	0·010	0·4	4·2	42·2	10·0	16	2·64	10·8	182	2·6	21·2
,, 21	16·6	46·5	0·010	0·5	3·3	34·0	10·3	15	2·27	7·6	216	3·1	24·5
Sept. 5	16·7	47·0	0·010	0·1	0·5	31·4	62·8	15	2·09	1·1	247	3·2	25·0
,, 20	16·9	46·9	0·010	0·2	−0·1	16·8	—	15	1·12	—	264	3·4	24·9
Oct. 6	17·1	52·4	0·010	0·2	5·5	35·0	6·4	16	2·19	11·7	299	3·6	30·4
,, 22	17·3	53·0	0·010	0·2	0·6	29·0	48·3	16	1·81	1·1	328	3·8	31·0
Nov. 6	17·4	52·1	0·010	0·1	−0·9	29·7	—	15	1·98	—	358	3·9	30·1

Table 18 : D3.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	14·0	26·5	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	14·0	25·0	0·009	nil	−1·5	9·3	—	14	0·66	—	9	nil	−1·5
June 9	14·4	28·5	0·010	0·4	3·5	24·6	7·0	14	1·76	14·0	34	0·4	2·0
,, 23	14·9	33·3	0·010	0·5	4·8	39·1	8·1	14	2·80	16·8	73	0·9	6·8
July 7	15·5	38·4	0·010	0·6	5·1	34·5	6·8	14	2·46	15·3	107	1·5	11·9
,, 21	16·3	49·0	0·011	0·8	10·6	44·8	4·2	14	3·20	27·6	152	2·3	22·5
Aug. 6	17·0	53·5	0·011	0·7	4·5	48·7	10·8	16	3·04	9·2	201	3·0	27·0
,, 21	17·4	54·5	0·011	0·4	1·0	32·9	32·9	15	2·19	1·9	234	3·4	28·0
Sept. 5	17·4	51·0	0·010	nil	−3·5	16·4	—	15	1·09	—	250	3·4	24·5

Table 19: D4.

Date (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	13·5	23·0	0·009	—	—	—	—	—	—	—	—	—	—
„ 26	13·5	21·5	0·009	nil	– 1·5	11·4	—	14	0·81	—	11	nil	–1·5
June 9	13·7	25·8	0·010	0·2	4·3	19·0	4·4	14	1·36	20·0	30	0·2	2·8
„ 23	14·0	27·0	0·010	0·3	1·2	20·9	17·4	14	1·49	4·6	51	0·5	4·0
July 7	14·3	29·8	0·010	0·3	2·8	21·3	7·6	14	1·52	10·4	73	0·8	6·8
„ 21	14·8	33·5	0·010	0·5	3·7	26·9	7·3	14	1·92	12·4	99	1·3	10·5
Aug. 6	15·5	39·7	0·010	0·7	6·2	35·6	5·7	16	2·22	18·5	135	2·0	16·7
„ 21	16·0	42·0	0·010	0·5	2·3	30·5	13·3	15	2·03	5·8	166	2·5	19·0
Sept. 5	16·4	44·5	0·010	0·4	2·5	30·3	12·1	15	2·02	6·0	196	2·9	21·5

Table 20: D5.

June 9	14·6	31·7	0·010	—	—	—	—	—	—	—	—	—	—
„ 23	15·0	33·2	0·010	0·4	1·5	21·9	14·6	14	1·56	4·7	22	0·4	1·5
July 7	15·4	34·8	0·010	0·4	1·6	18·9	11·8	14	1·35	4·8	41	0·8	3·1
„ 21	15·6	38·0	0·010	0·2	3·2	20·8	6·5	14	1·49	9·2	62	1·0	6·3
Aug. 6	16·0	41·0	0·010	0·4	3·0	34·3	11·4	16	2·14	8·0	96	1·4	9·4
„ 21	16·4	42·5	0·010	0·4	1·5	29·4	19·6	15	1·96	3·7	125	1·8	10·8
Sept. 5	16·8	47·2	0·010	0·4	4·7	34·1	7·3	15	2·27	11·1	159	2·2	15·5

Table 21: D6.

May 29	13·1	18·2	0·008	—	—	—	—	—	—	—	—	—	—
June 9	13·2	19·3	0·008	0·1	1·1	15·9	14·5	11	1·45	6·0	16	0·1	1·1
„ 23	13·4	20·5	0·009	0·2	1·2	22·6	18·8	14	1·61	6·2	38	0·3	2·3
July 7	13·7	21·6	0·008	0·3	1·1	16·5	15·0	14	1·18	5·3	55	0·6	3·4
„ 21	13·8	24·0	0·009	0·1	2·4	17·1	7·1	14	1·22	11·1	72	0·7	5·8
Aug. 6	14·3	26·0	0·009	0·5	2·0	31·8	15·9	16	1·99	8·3	104	1·2	7·8
„ 21	14·6	28·5	0·009	0·3	2·5	22·8	9·1	15	1·52	9·6	127	1·5	10·3
Sept. 5	15·1	33·0	0·010	0·5	4·5	32·4	7·2	15	2·16	15·8	159	2·0	14·8

TABLES 22–27.

Maximum Requirements and Growth of Female Plaice.

Table 22 : B1.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
June 17	14·7	26·7	0·008	—	—	—	—	—	—	—	—	—	—
„ 23	14·8	28·5	0·009	0·1	1·8	8·4	4·7	6	1·40	6·7	8	0·1	1·8
July 7	15·3	34·4	0·010	0·5	5·9	40·1	6·8	14	2·86	20·7	48	0·6	7·7
„ 21	15·8	43·0	0·011	0·5	8·6	40·2	4·7	14	2·87	25·0	89	1·1	16·3
Aug. 6	16·7	51·0	0·011	0·9	8·0	49·9	6·2	16	3·12	18·6	139	2·0	24·3
„ 21	17·4	58·5	0·011	0·7	7·5	52·2	7·0	15	3·48	14·7	191	2·7	31·8
Sept. 5	17·8	58·9	0·010	0·4	0·4	34·6	86·5	15	2·31	0·7	225	3·1	32·2
„ 20	17·9	59·5	0·010	0·1	0·6	30·1	50·2	15	2·01	1·0	255	3·2	32·8
Oct. 6	18·0	61·6	0·011	0·1	2·1	34·8	16·6	16	2·17	3·5	290	3·3	34·9
„ 22	18·1	55·7	0·010	0·1	−5·9	19·2	—	16	1·20	—	309	3·4	29·0
Nov. 6	18·0	55·7	0·010	−0·1	nil	21·6	—	15	1·4	—	331	3·3	29·0

Table 23 : B2.

June 17	15·5	30·5	0·008	—	—	—	—	—	—	—	—	—	—
„ 23	15·5	30·0	0 008	nil	−0·5	3·8	—	6	0·63	—	4	nil	−0·5
July 7	15·6	32·2	0·008	0·1	2·2	18·5	8·4	14	1·32	6·7	22	0·1	1·7
„ 21	16·1	42·5	0·010	0·5	10·3	49·9	4·8	14	3·56	32·0	72	0·6	12·0
Aug. 6	16·7	48·3	0·010	0·6	5·8	50·9	8·8	16	3·18	13·6	123	1·2	17·8
„ 21	17·2	54·5	0·011	0·5	6·2	46·5	7·5	15	3·10	12·8	170	1·7	24·0
Sept. 5	17·5	55·8	0·010	0·3	1·3	40·0	30·8	15	2·67	2·4	210	2·0	25·3
„ 20	17·4	60·4	0·011	−0·1	4·6	42·1	9·2	15	2·81	8·2	252	1·9	29·9
Oct. 6	18·0	63·5	0·011	0·6	3·1	43·1	13·9	16	2·69	5·1	295	2·5	33·0
„ 22	18·2	63·7	0·011	0·2	0·2	28·4	142·0	16	1·77	0·3	323	2·7	33·2
Nov. 6	18·2	64·1	0·011	nil	0·4	28·9	72·2	15	1·93	0·6	352	2·7	33·6

Table 24 : B3.

May 26	12·9	19·7	0·009	—	—	—	—	—	—	—	—	—	—
June 9	13·5	24·1	0·010	0·6	4·4	29·1	6·6	14	2·08	22·3	29	0·6	4·4
„ 23	14·7	30·0	0·009	1·2	5·9	31·6	5·4	14	2·26	24·5	61	1·8	10·3
July 7	15·3	35·3	0·010	0·6	5·3	34·4	6·5	14	2·46	17·7	95	2·4	15·6
„ 21	15·5	36·5	0·010	0·2	1·2	18·2	15·2	14	1·30	3·4	113	2·6	16·8
Aug. 6	15·9	38·8	0·010	0·4	2·3	28·9	12·6	16	1·81	6·3	142	3·0	19·1
„ 21	16·3	42·0	0·010	0·4	3·2	28·7	9·0	15	1·91	8·2	171	3·4	22·3
Sept. 5	16·5	43·8	0·010	0·2	1·8	26·2	14·6	15	1·75	4·3	197	3·6	24·1
„ 20	—	—								x			
Oct. 6	17·2	48·4	0·010	0·7	4·6	49·8	10·8	32	1·56	x	247	4·3	28·7
„ 22	17·4	49·7	0·009	0·2	1·3	23·8	18·3	16	1·49	2·7	271	4·5	30·0
Nov. 6	17·3	50·3	0·010	−0·1	0·6	25·1	41·8	15	1·67	1·2	296	4·4	30·6

Table 25 : B5.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (c.m.)	Cumulative growth. Wt. (gm)
May 26	15·5	34·5	0·009	—	—	—	—	—	—	—	—	—	—
June 9	15·9	42·5	0·011	0·4	8·0	38·7	4·8	14	2·76	23·2	39	0·4	8·0
„ 23	16·9	53·0	0·011	1·0	10·5	64·5	6·1	14	4·61	24·7	103	1·4	18·5
July 7	17·8	64·0	0·011	0·9	11·0	61·5	5·6	14	4·39	20·8	165	2·3	29·5
„ 21	18·1	69·0	0·012	0·3	5·0	41·5	8·3	14	2·96	7·8	206	2·6	34·5
Aug. 6	18·6	73·8	0·012	0·5	4·8	44·3	9·2	16	2·77	7·0	250	3·1	39·3
„ 21	19·2	82·5	0·012	0·6	8·7	47·8	5·5	15	3·12	1·2	298	3·7	48·0

Table 26 : B4.

May 26	12·3	17·2	0·009	—	—	—	—	—	—	—	—	—	—
June 9	13·0	22·4	0·010	0·7	5·2	26·6	5·1	14	1·90	30·2	27	0·7	5·2
„ 23	14·1	29·2	0·010	1·1	6·8	39·1	5·7	14	2·79	30·4	66	1·8	12·0
July 7	15·1	37·0	0·011	1·0	7·8	42·4	5·4	14	3·03	26·7	108	2·8	19·8
„ 21	15·9	42·0	0·010	0·8	5·0	38·3	7·7	14	2·74	13·5	146	3·6	24·8
Aug. 6	16·5	45·7	0·010	0·6	3·7	37·9	10·2	16	2·37	8·8	184	4·2	28·5
„ 21	16·5	47·5	0·011	nil	1·8	16·4	9·1	15	1·09	3·9	201	4·2	30·3
Sept. 5	16·5	43·8	0·010	nil	−3·7	11·3	—	15	0·75	—	212	4·2	26·6
„ 20	16·4	44·3	0·010	−0·1	0·5	11·3	22·6	15	0·75	1·1	232	4·1	27·1
Oct. 6	16·6	39·1	0·009	0·2	−5·2	32·7	—	16	2·04	—	256	4·3	21·9

Table 27 : B6.

May 12	14·3	27·5	0·009	—	—	—	—	—	—	—	—	—	—
„ 26	14·4	28·0	0·009	0·1	0·5	14·5	29·0	14	1·04	1·8	14	0·1	0·5
June 9	14·5	31·3	0·010	0·1	3·3	26·3	8·0	14	1·88	11·8	41	0·2	3·8
„ 23	15·0	34·0	0·010	0·5	2·7	31·5	11·7	14	2·25	8·6	72	0·7	6·5
July 7	15·2	37·2	0·011	0·2	3·2	27·7	8·7	14	1·98	9·4	100	0·9	9·7
„ 21	15·7	42·5	0·011	0·5	5·3	31·8	6·0	14	2·28	14·3	132	1·4	15·0
Aug. 6	16·2	46·0	0·011	0·5	3·5	35·9	10·3	16	2·24	8·2	168	1·9	18·5
„ 21	16·4	48·5	0·011	0·2	2·5	24·0	9·6	15	1·60	5·4	192	2·1	21·0
Sept. 5	16·5	48·2	0·011	0·1	−0·3	27·6	—	15	1·84	—	219	2·2	20·7
„ 20	16·4	45·6	0·010	−0·1	−2·6	13·8	—	15	0·92	—	233	2·1	18·1

TABLE 28.

Summarised Data Concerning Maximum Requirements and Growth in Male and Female (M) Plaice.

Fish No.	Sex.	Initial size.		Final size.		Growth.		Period.		Days in Period.	Total food per the period. (gm.)	Average weight of food per day for the period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.
		Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	From	To				
M1	♂	15·4	34·0	21·3	117·7	5·9	83·7	12 : 5	6 : 11	178	987	5·5	11·8
M2	♂	15·0	33·7	19·6	79·7	4·6	46·0	2 : 6	6 : 11	157	655	4·1	14·7
M3	♀	15·0	35·0	19·4	82·4	4·4	47·4	14 : 5	6 : 11	176	900	5·1	19·0
M4	♀	13·4	23·3	17·6	59·2	4·2	35·9	14 : 5	6 : 11	176	778	4·4	21·7

TABLES 29 AND 30.

MAXIMUM REQUIREMENTS AND GROWTH OF MALE (M) PLAICE.

TABLE 29: M1.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.^{3}	Growth per period. Lth. (cm)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	15·4	34·0	0·009	—	—	—	—	—	—	—	—	—	—
,, 26	15·8	41·5	0·009	0·4	7·5	50·2	6·7	14	3·6	22·1	50	0·4	7·5
June 9	16·8	49·8	0·010	1·0	8·3	64·1	7·7	14	4·6	20·0	114	1·4	15·8
,, 23	17·0	50·7	0·010	0·2	0·9	65·6	72·9	14	4·7	1·8	180	1·6	16·7
July 7	17·5	61·0	0·011	0·5	10·3	157·6	15·3	14	11·2	20·3	337	2·1	27·0
,, 21	17·9	65·0	0·011	0·4	4·0	81·4	20·4	14	5·8	6·6	419	2·5	31·0
Aug. 6	18·5	74·0	0·012	0·6	9·0	76·4	8·5	16	4·8	13·8	495	3·1	40·0
,, 21	19·3	85·0	0·012	0·8	11·0	82·5	7·5	15	5·5	14·9	578	3·9	51·0
Sept. 20	20·0	89·6	0·011	0·7	4·6	142·5	31·0	30	4·7	2x	720	4·6	55·6
Oct. 6	20·4	102·2	0·012	0·4	12·6	92·0	7·3	16	5·8	14·1	812	5·0	68·2
,, 23	20·7	102·0	0·012	0·3	−0·2	91·0	—	17	5·4	—	903	5·3	68·0
Nov. 6	21·3	117·7	0·012	0·6	15·7	84·0	5·4	14	6·0	15·4	987	5·9	83·7

TABLE 30: M2.

June 2	15·0	33·7	0·010	—	—	—	—	—	—	—	—	—	—
,, 9	15·0	31·9	0·009	nil	−1·8	15·7	—	7	2·2	—	16	nil	−1·8
,, 23	15·1	33·4	0·010	0·1	1·5	38·2	25·4	14	2·7	4·7	54	0·1	−0·3
July 7	15·5	36·0	0·010	0·4	2·6	66·3	25·5	14	4·7	7·8	120	0·5	2·3
,, 21	15·7	40·0	0·010	0·2	4·0	56·7	14·2	14	4·1	11·1	177	0·7	6·3
Aug. 6	16·4	44·7	0·010	0·7	4·7	69·2	14·7	16	4·3	11·7	246	1·4	11·0
,, 21	16·9	49·0	0·010	0·5	4·3	60·0	14·0	15	4·0	9·2	306	1·9	15·3
Sept. 20	17·8	57·4	0·010	0·9	8·4	111·8	13·3	30	3·7	2x	418	2·8	23·7
Oct. 6	18·3	62·2	0·010	0·5	4·8	86·4	−18·0	16	5·4	−8·4	504	3·3	28·5
,, 23	19·0	71·4	0·010	0·7	9·2	78·0	8·5	17	4·6	14·8	582	4·0	37·7
Nov. 6	19·6	79·7	0·011	0·6	8·3	72·0	8·7	14	5·1	11·6	654	4·6	46·0

TABLES 31 AND 32.

MAXIMUM REQUIREMENTS AND GROWTH OF FEMALE (M) PLAICE.

TABLE 31 : M3.

Date. (1930)	Size of fish.		Wt./ /Lth.^{3}	Growth per period.		Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)		Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt. (gm.)
May 14	15·0	35·0	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	15·1	34·8	0·010	0·1	– 0·2	36·0	—	12	3·0	—	36	0·1	– 0·2
June 9	15·5	39·8	0·011	0·4	5·0	55·7	11·1	14	4·0	14·4	92	0·5	4·8
,, 23	16·4	48·8	0·011	0·9	9·0	75·6	8·4	14	5·4	22·6	167	1·4	13·8
July 7	16·8	51·2	0·011	0·4	2·4	153·9	64·1	14	11·0	4·9	321	1·8	16·2
,, 21	17·2	59·0	0·011	0·4	7·8	83·9	10·8	14	6·0	15·2	405	2·2	24·0
Aug. 6	17·6	61·0	0·011	0·4	2·0	73·7	36·8	16	4·6	3·4	479	2·6	26·0
,, 21	18·0	65·0	0·011	0·4	4·0	63·5	15·9	15	4·2	6·6	542	3·0	30·0
Sept. 20	18·6	69·6	0·011	0·6	4·6	119·3	25·9	30	4·0	2x	662	3·6	34·6
Oct. 6	18·8	74·0	0·011	0·2	4·4	88 –	20·0	16	5·5	6·3	750	3·8	39·0
,, 23	19·0	76·2	0·011	0·2	2·2	78·0	35·5	17	4·6	3·0	828	4·0	41·2
Nov. 6	19·4	82·4	0·011	0·4	6·2	72·0	11·6	14	5·1	8·1	900	4·4	47·4

TABLE 32 : M4.

May 14	13·4	23·3	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	13·6	25·2	0·010	0·2	1·9	39·0	20·5	12	3·2	8·1	39	0·2	1·9
June 9	14·2	28·0	0·010	0·6	2·8	57·4	20·5	14	4·1	11·1	96	0·8	4·7
,, 23	14·3	28·7	0·010	0·1	0·7	72·3	103·3	14	5·2	2·5	169	0·9	5·4
July 7	14·7	31·8	0·010	0·4	3·1	70·6	22·8	14	5·0	10·8	239	1·3	8·5
,, 21	15·0	36·5	0·010	0·3	4·7	54·5	11·6	14	3·9	14·8	294	1·6	13·2
Aug. 6	15·1	36·0	0·010	0·1	– 0·5	60·4	—	16	3·8	—	354	1·7	12·7
,, 21	15·8	44·2	0·010	0·7	8·2	68·5	8·4	15	4·6	22·8	423	2·4	20·9
Sept. 20	16·8	50·6	0·011	1·0	6·4	117·5	18·4	30	3·9	2x	540	3·4	27·3
Oct. 6	17·0	52·6	0·011	0·2	2·0	87·5 –	43·7	16	5·5	4·0	628	3·6	29·3
,, 23	17·3	54·2	0·011	0·3	1·6	78·0	48·7	17	4·6	3·0	706	3·9	30·9
Nov. 6	17·6	59·2	0·011	0·3	5·0	72·8	14·6	14	5·2	9·2	778	4·2	35·9

TABLE 33.

Summarised Data Concerning Growth and Intermediate Food Supplies in Male and Female (I) Plaice.

Fish No.	Sex.	Initial size.		Final size.		Growth.		Period.		Days in Period.	Total food per the period. (gm.)	Average weight of food per day for the period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.
		Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	From	To				
I1	♂	15·7	33·7	19·2	65·7	3·5	32·0	13 : 6	6 : 11	146	217	1·5	6·8
I2	♂	15·0	32·0	17·6	56·0	2·6	24·0	12 : 5	6 : 11	178	252	1·4	10·5
I3	♀	12·8	20·3	17·6	59·5	4·8	39·2	14 : 5	6 : 11	176	247	1·4	6·3
I4	♀	14·9	32·0	18·3	67·4	3·4	35·4	18 : 6	6 : 11	141	208	1·5	5·9

TABLES 34 AND 35.

INTERMEDIATE RATIONS AND GROWTH OF MALE (I) PLAICE.

TABLE 34 : I1.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percent age growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
June 13	15·7	33·7	0·009	—	—	—	—	—	—	—	—	—	—
,, 23	15·9	36·3	0·009	0·2	2·6	12·8	4·9	10	1·28	7·7	13	0·2	2·6
July 7	16·3	39·3	0·009	0·4	3·0	14·0	4·7	14	1·00	8·3	27	0·6	5·6
,, 21	16·5	43·5	0·009	0·2	4·2	17·6	4·2	14	1·26	10·7	44	0·8	9·8
Aug. 6	17·1	48·0	0·009	0·6	4·5	26·0	5·8	16	1·62	10·3	70	1·4	14·3
,, 21	17·7	52·0	0·009	0·6	4·0	24·0	6·0	15	1·60	8·3	94	2·0	18·3
Sept. 19	18·2	57·6	0·010	0·5	5·6	48·0	8·6	29	1·66	2x	142	2·5	23·9
Oct. 6	18·8	64·3	0·010	0·6	6·7	32·0	4·8	17	1·88	11·6	174	3·1	30·6
,, 23	19·1	64·7	0·010	0·3	0·4	25·0	62·5	17	1·47	0·6	199	3·4	31·0
Nov. 6	19·2	65·7	0·010	0·1	1·0	18·0	18·0	14	1·29	1·5	217	3·5	32·0

TABLE 35 : I2.

May 12	15·0	32·0	0·009	—	—	—	—	—	—	—	—	—	—
,, 26	15·2	33·7	0·009	0·2	1·7	14·7	8·6	14	1·05	5·3	15	0·2	1·7
June 9	15·2	31·0	0·009	nil	− 2·7	13·8	—	14	0·99	—	28	0·2	− 1·0
,, 23	15·9	38·8	0·010	0·7	7·8	13·7	1·8	14	0·98	25·1	42	0·9	6·8
July 7	16·2	39·8	0·010	0·3	1·0	14·0	14·0	14	1·00	2·6	56	1·2	7·8
,, 21	16·2	42·0	0·010	nil	2·2	16·3	7·4	14	1·16	5·5	72	1·2	10·0
Aug. 6	16·3	44·0	0·010	0·1	2·0	26·0	13·0	16	1·62	5·0	98	1·3	12·0
,, 21	16·6	46·8	0·010	0·3	2·8	23·5	8·4	15	1·57	6·4	122	1·6	14·8
Sept. 19	17·1	51·0	0·010	0·5	4·2	48·0	11·4	29	1·70	2x	170	2·1	19·0
Oct. 6	17·4	54·6	0·010	0·3	3·6	32·0	8·9	17	1·88	7·1	202	2·4	22·6
,, 23	17·6	55·4	0·010	0·2	0·8	25·6	32·0	17	1·51	1·5	228	2·6	23·4
Nov. 6	17·6	56·0	0·010	nil	0·6	24·2	40·3	14	1·73	1·1	252	2·6	24·0

TABLES 36 AND 37.

INTERMEDIATE RATIONS AND GROWTH OF FEMALE (I) PLAICE.

TABLE 36: I3.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./ /Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 14	12·8	20·3	0·010	—	—	—	—	—	—	—	—	—	—
„ 26	12·8	21·3	0·010	nil	1·0	10·9	10·9	12	0·91	4·9	11	nil	1·0
June 9	13·2	23·5	0·010	0·4	2·2	11·2	5·1	14	0·80	10·3	22	0·4	3·2
„ 23	13·7	26·0	0·010	0·5	2·5	11·2	4·5	14	0·80	10·6	33	0·9	5·7
July 7	13·9	28·0	0·010	0·2	2·0	11·2	5·6	14	0·80	7·7	44	1·1	7·7
„ 21	14·4	32·5	0·011	0·5	4·5	17·0	3·8	14	1·21	16·1	61	1·6	12·2
Aug. 6	15·0	37·8	0·011	0·6	5·3	26·0	4·9	16	1·62	16·3	87	2·2	17·5
„ 21	15·7	43·0	0·011	0·7	5·2	23·5	4·5	15	1·57	13·8	111	2·9	22·7
Sept. 19	16·6	50·0	0·011	0·9	7·0	48·0	6·9	29	1·66	2x	159	3·8	29·7
Oct. 6	17·1	56·1	0·011	0·5	6·1	32·0	5·2	17	1·88	12·2	191	4·3	35·8
„ 23	17·5	58·8	0·011	0·4	2·7	28·3	10·5	17	1·70	4·8	219	4·7	38·5
Nov. 6	17·6	59·5	0·011	0·1	0·7	28·0	40·0	14	2·00	1·2	247	4·8	39·2

TABLE 37: I4.

June 18	14·9	32·0	0·010	—	—	—	—	—	—	—	—	—	—
„ 23	14·9	30·9	0·010	nil	− 1·1	1·7	—	5	0·34	—	2	nil	− 1·1
July 7	15·1	34·0	0·010	0·2	3·1	13·9	4·5	14	0·99	10·0	16	0·2	2·0
„ 21	15·4	39·5	0·010	0·3	5·5	17·3	3·1	14	1·24	16·2	33	0·5	7·5
Aug. 6	15·9	42·0	0·010	0·5	2·5	26·0	10·4	16	1·62	6·3	59	1·0	10·0
„ 21	16·5	48·0	0·010	0·6	6·0	24·0	4·0	15	1·60	14·3	83	1·6	16·0
Sept. 19	17·2	55·3	0·011	0·7	7·3	48·0	6·6	29	1·66	2x	131	2·3	23·3
Oct. 6	17·7	62·7	0·011	0·5	7·4	30·3	4·1	17	1·78	13·4	161	2·8	30·7
„ 23	18·1	65·3	0·011	0·4	2·6	28·2	10·8	17	1·66	4·1	189	3·2	33·3
Nov. 6	18·3	67·4	0·011	0·2	2·1	18·5	8·8	14	1·32	3·2	208	3·4	35·4

TABLES 38–41.

Data from January–April Experiments on Maintenance (Lympstone).

Table 38 : L1w (♂).

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.	Wt. (gm.)
Jan. 6	17·6	52·8	—	—	—	—	—	—	—	—	—	—
,, 20	17·6	50·5	nil	– 2·3	5·7	14	0·41	0·008	5·7	14	nil	– 2·3
Feb. 3	17·6	50·2	nil	– 0·3	6·1	14	0·44	0·009	11·8	28	nil	– 2·6
,, 17	17·6	50·9	nil	0·7	8·5	14	0·61	0·012	21·3	42	nil	– 1·9
Mar. 3	17·7	51·8	0·1	0·9	12·6	14	0·90	0·018	33·9	56	0·1	– 1·0
,, 17	17·6	51·0	– 0·1	– 0·8	9·8	14	0·70	0·014	43·7	70	nil	– 1·8
,, 31	17·7	51·8	0·1	0·8	10·5	14	0·75	0·015	54·2	84	0·1	– 1·0
April 14	17·7	52·8	nil	1·0	10·5	14	0·75	0·014	64·7	98	0·1	nil
,, 28	17·7	51·6	nil	– 1·2	10·1	14	0·72	0·014	74·8	112	0·1	– 1·2

Table 39 : L4w (♂).

Jan. 6	21·1	78·8	—	—	—	—	—	—	—	—	—	—
,, 20	21·1	76·9	nil	– 1·9	10·9	14	0·78	0·010	10·9	14	nil	– 1·9
Feb. 3	21·1	77·6	nil	0·7	10·8	14	0·77	0·010	21·7	28	nil	– 1·2
,, 17	21·1	79·4	nil	1·8	13·6	14	0·97	0·011	35·3	42	nil	0·6
Mar. 3	21·2	79·3	nil	– 0·1	15·6	14	1·11	0·014	50·9	56	nil	0·5
,, 17	21·1	81·3	nil	2·0	15·4	14	1·10	0·014	64·3	70	nil	2·5
,, 31	21·1	79·6	nil	– 1·7	13·5	14	0·96	0·012	79·8	84	nil	0·8
April 14	20·9	81·8	– 0·2	2·2	15·4	14	1·10	0·014	95·2	98	– 0·2	3·0
,, 28	20·9	79·0	nil	– 2·8	14·3	14	1·02	0·013	109·5	112	– 0·2	0·2

Table 40 : L5w (♀).

Jan. 6	18·9	69·8	—	—	—	—	—	—	—	—	—	—
,, 20	18·9	69·6	nil	– 0·2	9·0	14	0·64	0·009	9·0	14	nil	– 0·2
Feb. 3	19·0	69·8	0·1	0·2	6·0	14	0·43	0·006	15·0	28	0·1	nil
,, 17	19·0	73·1	nil	3·3	16·1	14	1·15	0·016	31·1	42	0·1	3·3
Mar. 3	19·2	75·9	0·2	2·8	18·0	14	1·29	0·017	49·1	56	0·3	6·1
,, 17	19·5	76·3	0·3	0·4	14·0	14	1·00	0·013	63·1	70	0·6	6·5
,, 31	19·7	77·9	0·2	1·6	13·1	14	0·93	0·012	76·1	84	0·8	8·1
April 14	19·9	79·1	0·2	1·2	10·4	14	0·75	0·010	86·6	98	1·0	9·3
,, 28	20·0	78·2	0·1	– 0·9	9·9	14	0·71	0·009	96·5	112	1·1	8·4

Table 41 : L7w (♀).

Jan. 6	22·7	103·4	—	—	—	—	—	—	—	—	—	—
,, 20	22·7	101·2	nil	– 2·2	13·5	14	0·96	0·009	13·5	14	nil	– 2·2
Feb. 3	22·7	101·1	nil	– 0·1	12·0	14	0·86	0·009	25·5	28	nil	– 2·3
,, 17	22·7	102·4	nil	1·3	8·9	14	0·64	0·006	34·4	42	nil	– 1·0
Mar. 3	22·7	101·5	nil	– 0·9	11·4	14	0·81	0·008	45·8	56	nil	– 1·9
,, 17	22·7	100·2	nil	– 1·3	10·3	14	0·74	0·007	56·1	70	nil	– 3·2
,, 31	22·7	99·9	nil	– 0·3	11·4	14	0·81	0·008	67·5	84	nil	– 3·5
April 14	22·7	100·4	nil	0·5	11·9	14	0·85	0·008	79·4	98	nil	– 3·0
,, 28	22·7	97·6	nil	– 2·8	11·8	14	0·84	0·009	91·2	112	nil	– 5·8

TABLE 42.

SUMMARISED DATA CONCERNING MAINTENANCE REQUIREMENTS OF MALE AND FEMALE PLAICE DURING 1929 AND 1930 (JAN.–APRIL), (PARTLY FROM MY PREVIOUS PAPER).

Fish No.	Sex.	Initial size.		Final size.		Growth.		Period.		No. of days in period.	Average quantity of food per day for period.	Ratio Food per day/ /mid body-weight for period.	Year.
		Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	From	To				
L1	♂	17·0	50·3	17·6	54·1	0·6	3·8	22 : 8	25 : 11	95	0·90	0·017	1929
		17·6	52·8	17·7	51·6	0·1	—1·2	6 : 1	28 : 4	112	0·58	0·011	1930
L4b	♂	20·8	75·1	21·0	76·9	0·2	1·8	30 : 9	25 : 11	56	1·11	0·015	1929
		21·1	78·8	20·9	79·0	—0·2	0·2	6 : 1	28 : 4	112	0·98	0·012	1930
L5	♀	18·5	62·8	18·6	66·9	0·1	4·1	8 : 8	25 : 11	109	0·88	0·014	1929
		18·9	69·8	20·0	78·2	1·1	8·4	6 : 1	28 : 4	112	0·86	0·012	1930
L7	♀	22·3	104·8	22·7	100·6	0·4	—4·3	4 : 9	25 : 11	82	1·32	0·013	1929
		22·7	103·4	22·7	97·6	nil	—5·8	6 : 1	28 : 4	112	0·81	0·008	1930

TABLE 43.

SUMMARISED DATA SHOWING MAINTENANCE REQUIREMENTS DURING JANUARY–APRIL OF MALE AND FEMALE, "MINIMUM-FED" AND "MAXIMUM-FED" PLAICE.

Fish No.	Sex.	Initial size. Lth. (c.m)	Initial size. Wt. (gm.)	Final size. Lth. (cm.)	Final size. Wt. (gm.)	Growth. Lth. (cm.)	Growth. Wt. (gm.)	Period From	Period To	No. of days in period.	Average quantity of food per day for period.	Ratio Food per day/ /mid body-weight for period.	
L1w	♂	17·6	52·8	17·7	51·8	0·1	—1·0	6 : 1	3 : 3	56	0·61	0·012	MINIMUM FISH.
L4w	♂	21·1	78·8	21·1	79·3	nil	0·5	6 : 1	3 : 3	56	0·91	0·012	
L5w	♀	18·9	69·8	19·2	75·9	0·3	6·1	6 : 1	3 : 3	56	0·88	0·012	
L7w	♀	22·7	103·4	22·7	101·5	nil	—1·9	6 : 1	3 : 3	56	0·67	0·007	
L10w	♂	23·1	117·2	23·1	112·5	nil	—4·7	6 : 1	3 : 3	56	0·02	0·000	MAXIMUM FISH.
L12w	♂	22·0	105·3	22·0	101·7	nil	—3·6	6 : 1	3 : 3	56	0·01	0·000	
L13w	♀	19·2	68·3	19·2	66·3	nil	—2·0	6 : 1	3 : 3	56	0·02	0·000	
L15w	♀	23·1	132·7	23·2	130·3	0·1	—2·4	6 : 1	3 : 3	56	0·02	0·000	

TABLES 44–47.

Maximum Requirements of Male and Female Plaice (Jan.–April).

Table 44: L10w (♂).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
Jan. 6	23·1	117·2	0·010	—	—	—	—	—	—	—	—	—	—
,, 20	23·1	112·5	0·009	nil	− 4·7	7·0	—	14	0·50	—	7	nil	− 4·7
Feb. 3	23·1	111·0	0·009	nil	− 1·5	1·2	—	14	0·09	—	8	nil	− 6·2
,, 17	23·1	110·2	0·009	nil	− 0·8	1·5	—	14	0·11	—	10	nil	− 7·0
Mar. 3	23·1	112·5	0·009	nil	2·3	12·3	5·3	14	0·88	2·1	22	nil	− 4·7
,, 17	23·3	118·0	0·009	0·2	5·5	37·4	6·8	14	2·67	4·9	59	0·2	0·8
,, 31	23·5	123·8	0·009	0·2	5·8	46·4	8·0	14	3·31	4·9	106	0·4	6·6
April 14	23·7	129·3	0·009	0·2	5·5	55·6	10·1	14	3·97	4·4	162	0·6	12·1
,, 28	23·7	128·2	0·009	nil	− 1·1	62·5	—	14	4·46	—	224	0·6	11·0

Table 45: L12w (♂).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
Jan. 6	22·0	105·3	0·010	—	—	—	—	—	—	—	—	—	—
,, 20	22·0	102·1	0·010	nil	− 3·2	0·1	—	14	0·01	—	nil	nil	− 3·2
Feb. 3	22·0	100·4	0·009	nil	− 1·7	0·1	—	14	0·01	—	nil	nil	− 4·9
,, 17	22·0	100·0	0·009	nil	− 0·4	0·4	—	14	0·03	—	1	nil	− 5·3
Mar. 3	22·0	101·7	0·010	nil	1·7	10·5	6·2	14	0·75	1·7	11	nil	− 3·6
,, 17	22·0	103·7	0·010	nil	2·0	37·9	16·9	14	2·71	2·0	49	nil	− 1·6
,, 31	22·0	103·6	0·010	nil	− 0·1	15·8	—	14	1·13	—	65	nil	− 1·7
April 14	22·0	103·0	0·010	nil	− 0·6	17·3	—	14	1·24	—	82	nil	− 2·3
,, 28	22·3	111·5	0·010	0·3	8·5	27·3	3·2	14	1·95	8·2	109	0·3	6·2

Table 46: L13w (♀).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
Jan. 6	19·2	68·3	0·010	—	—	—	—	—	—	—	—	—	—
,, 20	19·2	66·3	0·009	nil	− 2·0	0·9	—	14	0·06	—	1	nil	− 2·0
Feb. 3	19·2	65·0	0·009	nil	− 1·3	1·0	—	14	0·07	—	2	nil	− 3·3
,, 17	19·2	64·0	0·009	nil	− 1·0	1·8	—	14	0·13	—	4	nil	− 4·3
Mar. 3	19·2	66·3	0·009	nil	2·3	12·6*	5·5	14	0·90	3·6	16	nil	− 2·0
,, 17	19·3	71·5	0·010	0·1	5·2	35·1	6·7	14	2·51	7·8	51	0·1	3·2
,, 31	19·5	74·0	0·010	0·2	2·5	40·1	16·1	14	2·87	3·5	92	0·3	5·7
April 14	19·5	75·5	0·010	nil	1·5	42·9	28·6	14	3·06	2·0	135	0·3	7·2
,, 28	19·8	80·8	0·010	0·3	5·3	47·5	9·0	14	3·39	7·0	182	0·6	12·5

Table 47: L15w (♀).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
Jan. 6	23·1	132·7	0·011	—	—	—	—	—	—	—	—	—	—
,, 20	23·1	129·5	0·011	nil	− 3·2	3·0	—	14	0·21	—	3	nil	− 3·2
Feb. 3	23·2	128·0	0·010	0·1	− 1·5	0·9	—	14	0·06	—	4	0·1	− 4·7
,, 17	23·2	126·0	0·010	nil	− 2·0	1·2	—	14	0·09	—	5	0·1	− 6·7
Mar. 3	23·2	130·3	0·010	nil	4·3	12·5†	2·9	14	0·89	3·4	18	0·1	− 2·4
,, 17	23·5	135·1	0·010	0·3	4·8	39·4	8·2	14	2·81	3·7	57	0·4	2·4
,, 31	23·5	138·8	0·011	nil	3·7	45·1	12·2	14	3·22	2·7	102	0·4	6·1
April 14	23·5	152·4	0·012	nil	13·6	43·6	3·2	14	3·11	9·8	146	0·4	19·7
,, 28	23·7	128·8	0·010	0·2	−23·6‡	46·6	—	14	3·33	—	192	0·6	− 3·9

* May be 10·3. 1 daily ration of doubtful value. † May be 10·4. See footnotes to Table 46.
‡ Spawned during this period.

TABLES 48–51.

Maintenance Requirements of Male Plaice.

Table 48: L1.

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt (gm.)
May 12	17·9	62·2	—	—	—	—	—	—	—	—	—	—
,, 26	18·0	59·5	0·1	– 2·7	6·5	14	0·47	0·008	6·5	14	0·1	– 2·7
June 9	18·0	59·9	nil	0·4	9·9	14	0·71	0·012	16·4	28	0·1	– 2·3
,, 23	18·0	59·3	nil	– 0·6	9·9	14	0·71	0·012	26·3	42	0·1	– 2·9
July 7	18·0	59·0	nil	– 0·3	10·6	14	0·76	0·013	36·9	56	0·1	– 3·2
,, 21	18·0	57·3	nil	– 1·7	10·6	14	0·76	0·013	47·5	70	0·1	– 4·9
Aug. 4	18·0	57·7	nil	0·4	12·4	14	0·89	0·015	59·9	84	0·1	– 4·5
,, 18	18·0	58·0	nil	0·3	12·2	14	0·87	0·015	72·1	98	0·1	– 4·2
Sept. 1	18·0	56·5	nil	– 1·5	11·6	14	0·83	0·015	83·7	112	0·1	– 5·7
,, 15	18·0	56·8	nil	0·3	19·7	14	1·41	0·025	103·4	126	0·1	– 5·4
,, 29	18·1	57·1	0·1	0·3	12·7	14	0·91	0·016	116·1	140	0·2	– 5·1
Oct. 13	18·1	56·1	nil	– 1·0	12·6	14	0·90	0·016	128·7	154	0·2	– 6·1
,, 27	18·1	55·9	nil	– 0·2	13·7	14	0·98	0·017	142·4	168	0·2	– 6·3
Nov. 10	18·1	57·5	nil	1·6	13·9	14	0·98	0·017	156·3	182	0·2	– 4·7
,, 24	18·1	59·2	nil	1·7	12·3	14	0·88	0·015	168·6	196	0·2	– 3·0

Table 49: L2.

May 26	20·0	65·0	—	—	—	—	—	—	—	—	—	—
June 9	20·0	64·8	nil	– 0·2	11·9	14	0·85	0·013	20·2	14	nil	– 0·2
,, 23	20·0	64·3	nil	– 0·5	11·9	14	0·85	0·013	32·1	28	nil	– 0·7
July 7	20·0	62·2	nil	– 2·1	12·6	14	0·90	0·014	44·7	42	nil	– 2·8
,, 21	20·0	60·1	nil	– 2·1	14·2	14	1·01	0·017	58·9	56	nil	– 4·9
Aug. 4	20·0	60·2	nil	0·1	17·1	14	1·22	0·020	76·0	70	nil	– 4·8
,, 18	20·0	61·2	nil	1·0	16·9	14	1·21	0·020	92·9	84	nil	– 3·8
Sept. 1	20·0	60·0	nil	– 1·2	16·0	14	1·14	0·019	108·9	98	nil	– 5·0
,, 15	20·2	62·6	0·2	2·6	25·8	14	1·84	0·028	134·7	112	0·2	– 2·4
,, 29	20·2	63·8	nil	1·2	14·2	14	1·02	0·016	148·9	126	0·2	– 1·2
Oct. 13	20·2	65·1	nil	1·3	12·9	14	0·92	0·014	161·8	140	0·2	0·1
,, 27	20·2	69·5	nil	4·4	11·6	14	0·83	0·012	173·4	154	0·2	4·5
Nov. 10	20·2	70·3	nil	0·8	7·3	14	0·52	0·008	180·7	168	0·2	5·3
,, 24	20·2	70·1	nil	– 0·2	6·6	14	0·47	0·007	187·3	182	0·2	5·1

Table 50: L3.

Date (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	21·5	90·3	—	—	—	—	—	—	—	—	—	—
,, 26	21·5	83·5	nil	− 6·8	5·4	14	0·39	0·004	5·4	14	nil	− 6·8
June 9	21·5	82·4	nil	− 1·1	12·5	14	0·89	0·011	17·9	28	nil	− 7·9
,, 23	21·5	81·1	nil	− 1·3	15·1	14	1·08	0·013	33·0	42	nil	− 9·2
July 7	21·5	81·1	nil	nil	16·5	14	1·18	0·014	49·5	56	nil	− 9·2
,, 21	21·5	79·1	nil	− 2·0	16·5	14	1·18	0·014	66·0	70	nil	− 11·2
Aug. 4	21·5	79·8	nil	0·7	18·5	14	1·32	0·017	84·5	84	nil	− 10·5
,, 18	21·5	78·7	nil	− 1·1	17·9	14	1·28	0·016	102·4	98	nil	− 11·6
Sept. 1	21·5	77·8	nil	− 0·9	18·0	14	1·29	0·017	120·4	112	nil	− 12·5
,, 15	21·5	77·5	nil	− 0·3	21·2	14	1·51	0·019	141·6	126	nil	− 12·8
,, 29	21·6	74·4	0·1	− 3·1	14·4	14	1·03	0·014	156·0	140	0·1	− 15·9
Oct. 13	21·6	75·1	nil	0·7	14·8	14	1·06	0·014	170·8	154	0·1	− 15·2
,, 27	21·6	75·5	nil	0·4	14·1	14	1·01	0·013	184·9	168	0·1	− 14·8
Nov. 10	21·6	76·3	nil	0·8	12·6	14	0·90	0·014	197·5	182	0·1	− 14·0
,, 24	21·6	78·6	nil	2·3	11·8	14	0·84	0·011	209·3	196	0·1	− 11·7

Table 51: L4 (a and b).

(a)												
May 12	18·5	65·5	—	—	—	—	—	—	—	—	—	—
,, 26	18·5	64·8	nil	− 0·7	7·6	14	0·54	0·009	7·6	14	nil	− 0·7
June 9	18·5	63·8	nil	− 1·0	8·7	14	0·62	0·010	16·3	28	nil	− 1·7
,, 23	18·5	62·2	nil	− 1·6	9·9	14	0·71	0·011	26·2	42	nil	− 3·3
July 7	18·5	62·1	nil	− 0·1	11·5	14	0·82	0·013	37·7	56	nil	− 3·4
,, 21	18·5	62·5	nil	0·4	11·5	14	0·82	0·013	49·2	70	nil	− 3·0
Aug. 4	18·6	64·6	0·1	2·1	12·0	14	0·86	0·014	61·2	84	0·1	− 0·9
,, 18	18·6	63·5	nil	− 1·1	9·9	14	0·71	0·011	71·1	98	0·1	− 2·0
Sept. 1	18·7	63·0	0·1	− 0·5	11·1	14	0·79	0·012	82·2	112	0·2	− 2·5
(b)												
Sept. 15	24·4	149·6	—	—	—	—	—	—	—	—	—	—
,, 29	24·4	142·8	nil	− 6·8	9·4	14	0·67	0·005	9·4	14	nil	− 6·8
Oct. 13	24·4	145·1	nil	2·3	15·4	14	1·10	0·008	24·8	28	nil	− 4·5
,, 27	24·4	147·2	nil	2·1	13·2	14	0·94	0·006	38·0	42	nil	− 2·4
Nov. 10	24·6	147·2	0·2	nil	12·2	14	0·87	0·006	50·2	56	0·2	− 2·4
,, 24	24·7	149·5	0·1	2·3	12·3	14	0·88	0·006	62·5	70	0·3	− 0·1

TABLE 52.

SUMMARISED DATA : MAINTENANCE REQUIREMENTS OF MALE PLAICE.

Fish No.	Initial size.		Final size.		Growth.		Days in period.	Average weight of food per day.	Ratio Food per day/ /mid body-weight for period.
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)		(gm.)	
L1	17·9	62·2	18·1	57·5	0·2	—4·7	182	0·86	0·015
L2	20·0	65·0	20·2	70·3	0·2	5·3	168	1·03	0·015
L3	21·5	90·3	21·6	76·3	0·1	—14·0	182	1·09	0·013
L4 (a)	18·5	65·5	18·7	63·0	0·2	—2·5	112	0·74	0·012
L4 (b)	24·4	149·6	24·6	147·2	0·2	—2·4	56	0·89	0·006

TABLES 53–56.

Maintenance Requirements of Female Plaice.

Table 53 : L5.

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt. (gm.)
May 12	14·7	28·9	—	—	—	—	—	—	—	—	—	—
,, 26	14·7	28·6	nil	− 0·3	5·2	14	0·37	0·013	5·2	14	nil	− 0·3
June 9	14·7	27·8	nil	− 0·8	7·0	14	0·50	0·018	12·2	28	nil	− 1·1
,, 23	14·7	26·6	nil	− 1·2	7·8	14	0·56	0·021	20·0	42	nil	− 2·3
July 7	14·7	25·7	nil	− 0·9	9·9	14	0·71	0·027	29·9	56	nil	− 3·2
,, 21	14·7	25·3	nil	− 0·4	11·5	14	0·82	0·032	41·4	70	nil	− 3·6
Aug. 4	14·7	25·7	nil	0·4	12·0	14	0·86	0·034	53·4	84	nil	− 3·2
,, 18	14·7	25·9	nil	0·2	11·8	14	0·84	0·033	65·2	98	nil	− 3·0
Sept. 1	14·7	26·0	nil	0·1	11·6	14	0·83	0·032	76·8	112	nil	− 2·9
,, 15	14·7	26·1	nil	0·1	16·2	14	1·16	0·045	93·0	126	nil	− 2·8
,, 29	14·7	25·8	nil	− 0·3	11·0	14	0·79	0·031	104·0	140	nil	− 3·1
Oct. 13	14·7	27·7	nil	1·9	14·2	14	1·01	0·038	118·2	154	nil	− 1·2
,, 27	14·8	28·7	0·1	1·0	12·3	14	0·88	0·034	130·5	168	0·1	− 0·2
Nov. 10	14·8	29·1	nil	0·4	11·2	14	0·80	0·028	141·7	182	0·1	0·2
,, 24	14·9	31·3	0·1	2·2	10·9	14	0·78	0·026	152·6	196	0·2	2·4

Table 54 : L6 (a and b).

(a)												
May 12	18·0	69·9	—	—	—	—	—	—	—	—	—	—
,, 26	18·0	62·9	nil	− 7·0	6·1	14	0·44	0·007	6·1	14	nil	− 7·0
June 9	18·0	61·3	nil	− 1·6	10·7	14	0·76	0·012	16·8	28	nil	− 8·6
,, 23	18·0	59·8	nil	− 1·5	11·8	14	0·84	0·014	28·6	42	nil	− 10·1
July 7	18·0	61·1	nil	1·3	14·0	14	1·00	0·017	42·6	56	nil	− 8·8
,, 21	18·0	62·2	nil	1·1	12·4	14	0·89	0·013	55·0	70	nil	− 7·7
Aug. 4	18·0	63·8	nil	1·6	12·0	14	0·86	0·014	67·0	84	nil	− 6·1
,, 18	18·0	65·3	nil	1·5	10·5	14	0·75	0·012	77·5	98	nil	− 4·6
Sept. 1	18·0	64·6	nil	− 0·7	7·5	14	0·54	0·008	85·0	112	nil	− 5·3
(b)												
Sept. 29	17·3	56·2	—	—	—	—	—	—	—	—	—	—
Oct. 13	17·4	60·2	0·1	4·0	8·5	14	0·61	0·010	85	14	0·1	4·0
,, 27	17·6	64·3	0·2	4·1	4·6	14	0·33	0·005	13·1	28	0·3	8·1
Nov. 10	17·7	64·9	0·1	0·6	0·5	14	0·03	—	13·6	42	0·4	8·7
,, 24	17·8	67·7	0·1	2·8	3·0	14	0·21	0·003	16·6	56	0·5	11·5

Table 55 : L7.

Date (1930)	Size of fish.		Growth per period.		Weight of food per period. (gm.)	No. of days in period.	Average weight of food per day. (gm.)	Ratio of food per day/ /average body-wt. for period.	Total wt. of food to date. (gm.)	No. of days since commencement of expt.	Cumulative growth.	
	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)							Lth. (cm.)	Wt. (gm.)
June 23	18·0	58·5	—	—	—	—	—	—	—	—	—	—
July 7	18·1	57·1	0·1	− 1·4	11·2	14	0·80	0·014	11·2	14	0·1	− 1·4
„ 21	18·1	57·4	nil	0·3	12·6	14	0·90	0·016	23·8	28	0·1	− 1·1
Aug. 4	18·1	58·1	nil	0·7	12·6	14	0·90	0·016	36·4	42	0·1	− 0·4
„ 18	18·1	57·9	nil	− 0·2	9·9	14	0·71	0·012	46·3	56	0·1	− 0·6
Sept. 1	18·1	57·8	nil	− 0·1	10·1	14	0·72	0·012	56·4	70	0·1	− 0·7
„ 15	18·1	59·2	nil	1·4	19·4	14	1·39	0·024	75·8	84	0·1	0·7
„ 29	18·2	59·2	0·1	nil	8·8	14	0·63	0·011	84·6	98	0·2	0·7
Oct. 13	18·2	59·2	nil	nil	8·1	14	0·58	0·010	92·7	112	0·2	0·7
„ 27	18·2	60·2	nil	1·0	8·1	14	0·58	0·010	100·8	126	0·2	1·7
Nov. 10	18·2	60·4	nil	0·2	7·1	14	0·51	0·008	107·9	140	0·2	1·9
„ 24	18·2	61·0	nil	0·6	7·0	14	0·50	0·008	114·9	154	0·2	2·5

Table 56 : L8.

May 12	18·8	56·8	—	—	—	—	—	—	—	—	—	—
„ 26	18·8	56·8	nil	nil	7·9	14	0·56	0·010	7·9	14	nil	nil
June 9	18·8	50·9	nil	− 5·9	8·7	14	0·62	0·012	16·6	28	nil	− 5·9
„ 23	18·8	51·3	nil	0·4	14·8	14	1·06	0·021	31·4	42	nil	− 5·5
July 7	18·8	50·8	nil	− 0·5	14·6	14	1·04	0·020	46·0	56	nil	− 6·0
„ 21	18·8	52·5	nil	1·7	15·1	14	1·08	0·021	61·1	70	nil	− 4·3
Aug. 4	18·8	52·6	nil	0·1	13·4	14	0·96	0·018	74·5	84	nil	− 4·2
„ 18	18·8	53·0	nil	0·4	13·4	14	0·96	0·018	87·9	98	nil	− 3·8
Sept. 1	18·8	53·0	nil	nil	13·0	14	0·93	0·018	100·9	112	nil	− 3·8
„ 15	18·8	54·7	nil	1·7	19·3	14	1·38	0·026	120·2	126	nil	− 2·1
„ 29	18·9	55·1	0·1	0·4	11·2	14	0·80	0·014	131·3	140	0·1	− 1·7
Oct. 13	18·9	55·1	nil	nil	8·7	14	0·62	0·011	140·9	154	0·1	− 1·7
„ 27	18·9	55·1	nil	nil	8·7	14	0·62	0·011	148·7	168	0·1	− 1·7
Nov. 10	18·9	55·8	nil	0·7	8·7	14	0·62	0·011	157·4	182	0·1	− 1·0
„ 24	18·9	57·8	nil	2·0	8·0	14	0·57	0·010	165·4	196	0·1	1·0

TABLE 57.

SUMMARISED DATA: MAINTENANCE REQUIREMENTS OF FEMALE PLAICE.

Fish No.	Initial size. Lth. (cm.)	Initial size. Wt. (gm.)	Final size. Lth. (cm.)	Final size. Wt. (gm.)	Growth. Lth. (cm.)	Growth. Wt. (gm.)	Days in period.	Average weight of food per day. (gm.)	Ratio Food per day/ /mid body-weight per period.
L5	14·7	28·9	14·8	29·1	0·1	0·2	182	0·78	0·027
L6 (a)	18·0	69·9	18·0	64·6	nil	—5·3	112	0·76	0·011
L6 (b)	17·3	56·2	17·7	64·9	0·4	8·7	42	0·32	0·005
L7	18·0	58·5	18·2	60·4	0·2	1·9	140	0·77	0·013
L8	18·8	56·8	18·9	55·8	0·1	—1·0	182	0·86	0·015

TABLE 58.

Summarised Data: Maximum Requirements and Growth of Male and Female Plaice.

Fish No.	Sex.	Initial size.		Final size.		Growth.		Period.		Days in Period.	Total food per the period. (gm.)	Average weight of food per day for the period (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.
		Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	Lth. (cm.)	Wt. (gm.)	From	To				
L9a	♂	18·4	67·4	18·9	78·3	0·5	10·9	12 : 5	23 : 6	42	126	3·0	11·6
L9b		21·2	92·5	22·1	104·5	0·9	12·0	27 : 6	10 : 11	136	545	4·0	45·4
L10a	♂	17·2	55·6	17·7	56·9	0·5	1·3	12 : 5	4 : 8	84	242	2·9	186·1
L10b		21·4	73·9	23·1	127·7	1·7	53·8	4 : 8	10 : 11	98	515	5·3	9·6
L11a	♂	21·8	101·2	22·7	118·8	0·9	17·6	12 : 5	1 : 9	112	469	4·2	26·6
L11b		22·6	120·1	23·8	160·8	1·2	40·7	29 : 9	10 : 11	42	267	6·4	6·5
L12a	♂	19·3	75·1	21·3	105·6	2·0	30·5	12 : 5	1 : 9	112	471	4·2	15·4
L12b		20·3	86·8	20·8	102·7	0·5	15·9	29 : 9	10 : 11	42	175	4·2	11·0
L13	♀	20·0	73·7	22·0	114·6	2·0	40·9	12 : 5	10 : 11	182	819	4·5	20·0
L14a	♀	17·0	50·5	20·6	97·8	3·6	47·3	12 : 5	1 : 9	112	546	4·9	11·5
L14b		25·1	173·2	26·1	205·3	1·0	32·1	15 : 9	10 : 11	56	409	7·3	12·7
L15	♀	20·1	75·7	22·1	115·7	2·0	40·0	12 : 5	10 : 11	182	896	4·9	22·4
L16a	♀	19·5	79·0	20·7	102·8	1·2	23·8	12 : 5	23 : 6	42	209	5·0	8·8
L16b		20·8	104·9	24·5	192·8	3·7	87·9	7 : 6	10 : 11	126	827	6·6	9·4

TABLES 59–62.

Maximum Requirements and Growth of Male Plaice.

Table 59: L9 (a and b).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.^{3}	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
(a)													
May 12	18·4	67·4	0·011	—	—	—	—	—	—	—	—	—	—
,, 26	18·5	69·0	0·011	0·1	1·6	21·1	13·2	14	1·51	2·4	21	0·1	1·6
June 9	18·8	77·6	0·012	0·3	8·6	63·2	7·3	14	4·51	12·5	84	0·4	10·2
,, 23	18·9	78·3	0·012	0·1	0·7	42 0	60·0	14	3·00	0·9	126	0·5	10·9
(b)													
June 27	21·2	92·5	0·010	—	—	—	—	—	—	—	—	—	—
July 7	21·3	92·6	0·010	0·1	0·1	48·0	480·0	10	4·80	0·1	48	0·1	0·1
,, 21	21·5	94·8	0·010	0·2	2·2	63·1	28·7	14	4·51	2·4	111	0·3	2·3
Aug. 4	21·2	96·6	0·010	− 0·3	1·8	56·9	31·6	14	4·06	1·9	168	nil	4·1
,, 18	21·7	101·2	0·010	0·5	4·6	60·9	13·2	14	4·35	4·8	229	0·5	8·7
Sept. 1	21·8	101·1	0·010	0·1	− 0·1	61·6	—	14	4·40	—	290	0·6	8·6
,, 15	21·9	95·9	0·009	0·1	− 5·2	38·2	—	14	2·73	—	329	0·7	3·4
,, 29	22·0	100·5	0·010	0·1	4·6	50·7	11·0	14	3·62	4·8	379	0·8	8·0
Oct. 13	22·0	103·8	0·010	nil	3·3	66·4	20·1	14	4·74	3·3	446	0·8	11·3
,, 27	22·1	108·1	0·010	0·1	4·3	59·1	13·7	14	4·22	4·1	505	0·9	15·6
Nov. 10	22·1	104·5	0·010	nil	− 3·6	40·5	—	14	2·89	—	545	0·9	12·0
,, 24	22·1	104·6	0·010	nil	0·1	20·1	201·0	14	1·44	0·1	565	0·9	12·1

Table 60: L10 (a and b).

(a)													
May 12	17·2	55·6	0·011	—	—	—	—	—	—	—	—	—	—
,, 26	17·2	53·8	0·011	nil	− 1·8	28·8	—	14	2·06	—	29	nil	− 1·8
June 9	17·4	57·7	0·011	0·2	3·9	65·5	16·8	14	4·68	7·2	94	0·2	2·1
,, 23	17·7	60·0	0·011	0·3	2·3	45·5	19·8	14	3·25	4·0	140	0·5	4·4
July 7	17·7	60·7	0·011	nil	0·7	41·9	59·9	14	2·99	1·2	182	0·5	5·1
,, 21	17·7	59·9	0·011	nil	− 0·8	38·4	—	14	2·74	—	220	0·5	4·3
Aug. 4	17·7	56·9	0·010	nil	− 3·0	20·8	—	14	1·49	—	241	0·5	1·3
(b)													
Aug. 4	21·4	73·9	0·008	—	—	—	—	—	—	—	—	—	—
,, 18	21·6	84·3	0·008	0·2	10·4	59·4	5·7	14	4·24	14·1	59	0·2	10·4
Sept. 1	21·8	89·8	0·009	0·2	5·5	68·8	12·5	14	4·91	6·5	128	0·4	15·9
,, 15	22·0	97·5	0·009	0·2	7·7	66·9	8·7	14	4·78	8·6	195	0·6	23·6
,, 29	22·3	107·7	0·010	0·3	10·2	81·8	8·0	14	5·84	10·5	277	0·9	33·8
Oct. 13	22·7	119·1	0·010	0·4	11·4	96·8	8·5	14	6·91	10·6	374	1·3	45·2
,, 27	22·9	123·0	0·010	0·2	3·9	83·1	21·3	14	5·94	3·3	457	1·5	49·1
Nov. 10	23·1	127·7	0·010	0·2	4·7	57·9	12·3	14	4·14	3·8	515	1·7	53·8
,, 24	23·2	129·6	0·010	0·1	1·9	28·6	15·1	14	2·04	1·5	543	1·8	55·7

Table 61 : L11 (a and b).

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
(a)													
May 12	21·8	101·2	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	21·9	100·2	0·010	0·1	− 1·0	40·2	—	14	2·87	—	40	0·1	− 1·0
June 9	21·9	96·6	0·009	nil	− 3·6	51·6	—	14	3·69	—	92	0·1	− 4·6
,, 23	22·0	102·3	0·010	0·1	5·7	59·1	10·4	14	4·22	5·9	151	0·2	1·1
July 7	22·1	105·0	0·010	0·1	2·7	60·8	22·5	14	4·34	2·6	212	0·3	3·8
,, 21	22·3	110·3	0·010	0·2	5·3	62·5	11·8	14	4·46	5·0	274	0·5	9·1
Aug. 4	22·4	114·6	0·010	0·1	4·3	62·6	12·2	14	4·47	3·9	337	0·6	13·4
,, 18	22·5	120·0	0·011	0·1	5·4	68·2	12·6	14	4·87	4·7	405	0·7	18·8
Sept. 1	22·7	118·8	0·010	0·2	− 1·2	61·9	—	14	4·42	—	467	0·9	17·6
(b)													
Sept. 29	22·6	120·1	0·010	—	—	—	—	—	—	—	—	—	—
Oct. 13	22·9	136·9	0·010	0·3	16·8	75·0	4·5	14	5·36	13·9	75	0·3	16·8
,, 27	23·5	158·5	0·012	0·6	21·6	103·3	4·8	14	7·36	15·8	178	0·9	38·4
Nov. 10	23·8	160·8	0·012	0·3	2·3	88·5	38·5	14	6·32	1·5	267	1·2	40·7
,, 24	23·9	166·2	0·012	0·1	5·4	45·1	8·4	14	3·23	3·4	312	1·3	46·1

Table 62 : L12 (a and b).

(a)													
May 12	19·3	75·1	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	19·6	82·3	0·011	0·3	7·2	51·6	7·2	14	3·69	9·6	52	0·3	7·2
June 9	20·0	86·9	0·011	0·4	4·6	64·7	14·1	14	4·62	5·6	116	0·7	11·8
,, 23	20·2	89·3	0·011	0·2	2·4	61·8	25·7	14	4·41	2·8	178	0·9	14·2
July 7	20·4	90·5	0·011	0·2	1·2	56·7	47·2	14	4·05	1·3	235	1·1	15·4
,, 21	20·5	95·1	0·011	0·1	4·6	55·0	12·0	14	3·93	5·1	290	1·2	20·0
Aug. 4	20·8	99·9	0·011	0·3	4·8	58·9	12·3	14	4·21	5·0	349	1·5	24·8
,, 18	21·1	104·3	0·011	0·3	4·4	63·3	14·4	14	4·52	4·4	412	1·8	29·2
Sept. 1	21·3	105·6	0·011	0·2	1·3	59·3	45·6	14	4·24	1·2	471	2·0	30·5
(b)													
Sept. 29	20·3	86·8	0·010	—	—	—	—	—	—	—	—	—	—
Oct. 13	20·4	92·1	0·011	0·1	5·3	61·0	11·5	14	4·36	6·1	61	0·1	5·3
,, 27	20·7	99·0	0·011	0·3	6·9	61·5	8·9	14	4·39	7·5	122	0·4	12·2
Nov. 10	20·8	102·7	0·011	0·1	3·7	52·3	14·1	14	3·74	3·7	175	0·5	15·9
,, 24	20·9	105·4	0·012	0·1	2·7	36·5	13·5	14	2·61	2·6	211	0·6	18·6

TABLES 63–66.

Maximum Requirements and Growth of Female Plaice.

Table 63: L13.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	20·0	73·7	0·009	—	—	—	—	—	—	—	—	—	—
,, 26	20·3	85·7	0·010	0·3	12·0	53·9	4·5	14	3·85	16·3	54	0·3	12·0
June 9	20·6	88·2	0·010	0·3	2·5	74·9	30·0	14	5·35	2·9	129	0·6	14·5
,, 23	20·7	94·6	0·011	0·1	6·4	71·7	11·2	14	5·12	7·3	200	0·7	20·9
July 7	20·8	100·2	0·011	0·1	5·6	69·3	12·4	14	4·95	5·9	270	0·8	26·5
,, 21	21·0	106·7	0·011	0·2	6·5	62·3	9·6	14	4·45	6·5	332	1·0	33·0
Aug. 4	21·3	109·1	0·011	0·3	2·4	66·0	27·5	14	4·71	1·3	398	1·3	35·4
,, 18	21·3	109·3	0·011	nil	0·2	66·1	330·0	14	4·72	0·2	464	1·3	35·6
Sept. 1	21·3	106·4	0·011	nil	−2·9	63·5	—	14	4·54	—	528	1·3	32·7
,, 15	21·4	107·0	0·011	0·1	0·6	48·4	80·6	14	3·46	0·6	576	1·4	33·3
,, 29	21·5	108·9	0·011	0·1	1·9	46·0	24·2	14	3·29	1·8	622	1·5	35·2
Oct. 13	21·7	120·0	0·012	0·2	11·1	77·9	7·0	14	5·56	10·2	700	1·7	46·3
,, 27	22·0	123·1	0·012	0·3	3·1	75·8	24·5	14	5·41	2·6	776	2·0	49·4
Nov. 10	22·0	114·6	0·011	nil	−8·5	45·5	—	14	3·25	—	821	2·0	40·9
,, 24	22·0	121·1	0·012	nil	6·5	22·4	3·4	14	1·60	5·7	844	2·0	47·4

Table 64: L14 (a and b).

(a)													
May 12	17·0	50·5	0·010	—	—	—	—	—	—	—	—	—	—
,, 26	17·8	66·8	0·012	0·8	16·3	40·9	2·5	14	2·92	32·1	41	0·8	16·3
June 9	18·7	78·0	0·012	0·9	11·2	77·9	7·0	14	5·56	16·8	119	1·7	27·5
,, 23	19·1	83·5	0·012	0·4	5·5	75·0	13·6	14	5·36	7·1	194	2·1	33·0
July 7	19·3	86·8	0·012	0·2	3·3	71·0	21·8	14	5·07	4·0	265	2·3	36·3
,, 21	19·6	93·4	0·012	0·3	6·6	74·4	11·3	14	5·32	7·6	339	2·6	42·9
Aug. 4	20·1	97·7	0·012	0·5	4·3	75·0	17·4	14	5·36	4·6	414	3·1	47·2
,, 18	20·3	97·1	0·012	0·2	−0·6	67·0	—	14	4·79	—	481	3·3	46·6
Sept. 1	20·6	97·8	0·011	0·3	0·7	66·5	95·0	14	4·75	0·7	548	3·6	47·3
(b)													
Sept. 15	25·1	173·2	0·011	—	—	—	—	—	—	—	—	—	—
,, 29	25·4	182·2	0·011	0·3	9·0	72·9	8·1	14	5·21	5·2	73	0·3	9·0
Oct. 13	25·9	200·4	0·012	0·5	18·2	115·0	6·3	14	8·21	10·0	188	0·8	27·2
,, 27	26·1	211·3	0·012	0·2	10·9	119·4	11·0	14	8·53	5·4	307	1·0	38·1
Nov. 10	26·1	205·3	0·012	nil	−6·0	101·8	—	14	7·27	—	409	1·0	32·1
,, 24	26·1	204·4	0·011	nil	−0·9	26·9	—	14	1·92	—	436	1·0	31·2

Table 65: L15.

Date. (1930)	Size of fish. Lth. (cm.)	Size of fish. Wt. (gm.)	Wt./Lth.³	Growth per period. Lth. (cm.)	Growth per period. Wt. (gm.)	Total food per period. (gm.)	Gm. of food per 1·0 gm. increase in weight of fish.	No. of days in period.	Average food per day during each period. (gm.)	Percentage growth in wt.	Total food to date (gm.)	Cumulative growth. Lth. (cm.)	Cumulative growth. Wt. (gm.)
May 12	20·1	75·7	0·009	—	—	—	—	—	—	—	—	—	—
,, 26	20·2	85·2	0·010	0·1	9·5	61·0	6·4	14	4·36	12·5	61	0·1	9·5
June 9	20·8	96·7	0·011	0·6	11·5	94·2	8·2	14	6·73	13·5	155	0·7	21·0
,, 23	21·0	102·1	0·011	0·2	5·4	86·4	16·0	14	6·17	5·6	242	0·9	26·4
July 7	21·1	106·5	0·011	0·1	4·4	68·8	15·6	14	4·91	4·3	310	1·0	30·8
,, 21	21·2	108·6	0·011	0·1	2·1	70·6	33·6	14	5·04	1·0	381	1·1	32·9
Aug. 4	21·5	111·6	0·011	0·3	3·0	71·0	23·7	14	5·07	2·8	452	1·4	35·9
,, 18	21·6	114·1	0·011	0·1	2·5	72·6	29·0	14	5·19	2·2	525	1·5	38·4
Sept. 1	21·6	110·4	0·011	nil	− 3·7	65·2	—	14	4·66	—	590	1·5	34·7
,, 15	21·7	111·3	0·011	0·1	0·9	60·4	67·1	14	4·31	0·8	650	1·6	35·6
,, 29	21·9	115·3	0·011	0·2	4·0	66·9	16·7	14	4·78	3·6	717	1·8	39·6
Oct. 13	22·1	118·7	0·011	0·2	3·4	77·3	22·7	14	5·52	3·0	794	2·0	43·0
,, 27	22·1	120·4	0·011	nil	1·7	57·4	33·8	14	4·10	1·4	852	2·0	44·7
Nov. 10	22·1	115·7	0·011	nil	− 4·7	44·0	—	14	3·14	—	896	2·0	40·0
,, 24	22·1	114·4	0·011	nil	− 1·3	20·9	—	14	1·49	—	917	2·0	38·7

Table 66: L16 (a and b).

(a)													
May 12	19·5	79·0	0·011	—	—	—	—	—	—	—	—	—	—
,, 26	19·8	88·5	0·011	0·3	9·5	51·7	5·4	14	3·69	12·0	52	0·3	9·5
June 9	20·5	99·3	0·011	0·7	10·8	84·4	7·8	14	6·03	12·3	136	1·0	20·3
,, 23	20·7	102·8	0·011	0·2	3·5	73·4	21·0	14	5·24	3·5	209	1·2	23·8
(b)													
July 7	20·8	104·9	0·012	—	—	—	—	—	—	—	—	—	—
,, 21	21·5	114·7	0·012	0·7	9·8	72·2	7·4	14	5·14	9·3	72	0·7	9·8
Aug. 4	22·0	126·4	0·012	0·5	11·7	82·2	7·0	14	5·87	10·2	154	1·2	21·5
,, 18	22·5	136·7	0·012	0·5	10·3	90·4	8·8	14	6·46	8·1	245	1·7	31·8
Sept. 1	22·9	142·3	0·012	0·4	5·6	89·0	15·9	14	6·36	4·1	334	2·1	37·4
,, 15	23·1	153·5	0·012	0·2	11·2	85·4	7·6	14	6·10	7·9	420	2·3	48·6
,, 29	23·6	158·7	0·012	0·5	5·2	76·4	14·7	14	5·46	3·4	496	2·8	53·8
Oct. 13	24·0	177·7	0·013	0·4	19·0	121·5	6·4	14	8·68	12·0	618	3·2	72·8
,, 27	24·3	187·0	0·013	0·3	9·3	106·2	11·4	14	7·59	5·2	724	3·5	82·1
Nov. 10	24·5	192·8	0·013	0·2	5·8	98·2	16·9	14	7·01	3·1	822	3·7	87·9
,, 24	24·6	201·8	0·014	0·1	9·0	67·5	7·5	14	4·82	4·6	890	3·8	96·9

A Statistical Study of Growth and Maintenance in the Plaice (*Pleuronectes platessa* L.).

By

Ben Dawes, A.R.C.S., D.I.C., F.L.S.

With 5 Figures in the Text.

CONTENTS.

		PAGE
I.	Introduction and Methods	949
	Results	951
II.	Size Data	951
III.	Growth Data	952
IV.	Food Data	953
V.	Correlation between Food and Growth	954
VI.	Lympstone Winter; L.W. Experiments	963
VII.	Notation used	965
VIII.	Summary	965
IX.	References	966
X.	Appendix, Tables 8–16	967—975

I. Introduction and Methods.

The data for this paper were obtained from experiments carried out at Cawsand and Lympstone under the auspices of the Marine Biological Association during the years 1929–30 and subject to the direction of Dr. E. J. Allen, F.R.S. The aim of the experiments was to determine the maintenance requirements and growth efficiency of plaice during the third season of growth, and to ascertain what fraction of total food taken by such fish is available for growth promotion. To some extent this aim has already been realised, for in two previous papers (1 and 2) many data have been presented and discussed and interesting conclusions bearing upon growth and maintenance have been put forward. To these papers the reader is referred also for a full description of the experimental methods employed and the practical difficulties encountered. In one important respect, however, the results of the work remain incompletely expressed due to the purely arithmetic mode of treatment of the data, which remain in rather unwieldy form and show a heterogeneity entirely due to variable

performances. In the first instance it was essential to emphasise this variability, but ultimately the indices of growth efficiency and maintenance must relate to populations rather than to individuals if they are to be of maximum assistance to fishery research. The aim of the present paper is to remedy this deficiency by applying appropriate statistical treatment to the data, thus enabling them to be presented in more compact and more satisfactory form. At the outset it is desired to thank Mr. T. Edser of the "S" Branch of the Ministry of Agriculture and Fisheries for his suggestion that the work be undertaken and for invaluable assistance willingly rendered.

A glance at my previous papers will serve to show that the experiments were divided up into periods of approximately a fortnight in duration, so that each period may be regarded as a separate experiment yielding definite results. In the present paper, it is intended to consider these periods as units, after reducing by simple proportion where necessary to periods of 14 days. Larger masses of data are thereby rendered available and can be dealt with statistically ; the individual is the plaice used in one unit period of 14 days. Weight increase (to be expressed in grammes) and food taken (to be expressed as a percentage of the initial body-weight) are the only growth characteristics utilised, and factors such as temperature are of necessity ignored, except in as far as the mean temperature differs from one place to another and from one year to the next. The aim of the work may be more closely defined as being to determine the maintenance requirements and growth efficiency of the average member of a plaice community during the "average" fortnight of the third season of growth.

In setting out the data initially, weight changes were considered to the nearest 0·1 gm., food quantities to the nearest 1 per cent, but in compiling the tables, grouping was effected into 1·0 gm. and in 10 per cent classes, except in the case of the L.W. experiments, where 1·0 gm. and 5 per cent classes were formed. Males and females were considered separately in order to ascertain the possibility of marked differences in degree of correlation or in steepness of the regression lines, and Cawsand and Lympstone results were kept separate for similar reasons. All calculations have been made by the use of four-figure logarithms, have been carried out twice independently, and then finally checked. In my previous papers many scraps of data were omitted for the sake of brevity since they did not materially affect conclusions formed, but in the present paper all available data have received due consideration. It is usual in work of this kind to reject extreme variates following upon the use of Chauvenet's criterion and prior to calculation of statistical characteristics, but in this case suspected extreme variates are relatively numerous and it was deemed advisable to let them stand.

RESULTS.

II. Size Data.

The primary consideration of defining as closely as possible the size limits of and the distribution of sizes in the various statistical populations is made in Table 1, where relevant summarised size data are shown.

TABLE 1.

Summarised Body-Weight Data Defining the Statistical Populations.

Population.	Sex.	No. of Fish.	Weight Distribution (gm.). Lower Limit	Lower Quartile Q1.	Median Q2.	Upper Quartile Q3.	Upper Limit.	Arithmetic Mean (gm.) $\bar{X} \pm \frac{\sigma_x}{\sqrt{n}}$	Standard Deviation (gm.) $\pm \sigma_x \pm \frac{\sigma_x}{\sqrt{2n}}$
C1929	♂	175	13	21·7	29·9	43·2	105	35·2±1·3	18·0±1·0
,,	♀	189	15	27·0	38·6	57·2	105	44·6±1·6	22·0±1·1
C 1930	♂	181	18	33·9	46·4	64·0	118	52·7±1·8	24·2±1·3
,,	♀	194	17	32·5	47·3	61·6	141	54·1±2·1	29·5±1·5
L 1929	♂	117	21	57·7	79·0	109·8	159	84·0±3·0	32·1±2·1
,,	♀	111	27	61·1	74·2	112·7	186	90·4±4·0	41·7±2·8
L 1930	♂	119	54	63·4	79·8	102·8	166	86·7±2·5	27·7±1·8
,,	♀	119	25	59·2	73·1	109·6	211	86·1±4·1	44·8±2·9

Under the heading of " Weight Distribution " lower and upper size limits are indicated, as are also lower, median, and upper quartiles, these being the sizes below which 25, 50, and 75 per cent of the variates occur in each population, and therefore merely convenient indices of the grouping of each 25 per cent of the variates. The outstanding feature of the distributions is that they are not normal, the median being far below the mid-point of the total range. The populations thus include a majority of small individuals, the sampling among large individuals being more than merely correspondingly inferior in view of the extended distribution above the upper quartile. It is also noticeable that the quartiles of the Lympstone populations are fairly uniform and dissimilar to those of the Cawsand populations. One would be inclined to expect that since the populations are thus rendered statistically distinct, this distinction would be reflected into subsequent correlative findings, as the unit of growth is 1·0 gm., that of food 1 per cent of the body-weight at the outset of the experimental period.

The peculiarity of the distributions mentioned above is emphasised by the differences between the medians and the means, $\frac{1}{n}\Sigma(f.X)$, as is shown in Table 1. The means are in every instance considerably greater than the medians. When probable errors are taken into account, the means are fairly uniform as regards sex, but clearly distinct in the C and L groups, even though generally speaking all plaice considered are two-year-olds.

The most convenient measure of dispersion is the standard deviation,

and since this index is to be used consistently throughout the present paper it is essential first of all to define it as the "square-root of the arithmetic mean of the squares of all deviations, deviations being measured from the arithmetic mean of the observations" (Yule, **4**, p. 134). Here, with its standard error, it is

$$\sigma_x = \sqrt{\tfrac{1}{n}\Sigma(f.D^2)} \pm \frac{\sigma_x}{\sqrt{2n}}$$

It can be converted readily into the mean deviation, $\frac{1}{n}(f.D)$ irrespective of the sign of D, by multiplying by ·7979, the standard error into the probable error of the determination by multiplying by ·6745. The meaning of the index has been admirably illustrated by Mr. Ford (Ford,* **3**, p. 256).

In Table 1 it is seen that, with the exception of the L. 1930 ♂ population, standard deviations happen to form an ascending series from the top of the table to the bottom. The degree of dispersion of body-weights is thus greater in L. than in C. groups, as in the case of means.

It is to be observed, however, that the degree of dispersion is not a reliable index of growth, since maintained individuals of all sizes are included in the populations.

III. Growth Data.

Examination of the columns under the heading "Distribution of Weight Changes" in Table 2 will serve to indicate that in all populations

TABLE 2.

Growth Data.

Population.	Sex.	No. of Fish.	Distribution of Weight Changes (gm.). Lower Limit.	Lower Quartile Q1.	Median Q2.	Upper Quartile Q3.	Upper Limit.	Mean Growth (gm.) $\left(\bar{x} \pm \frac{\sigma}{\sqrt{n}}\right)$	Standard Deviation (gm.) $\left(\pm\sigma_x \pm \frac{\sigma_x}{\sqrt{2n}}\right)$
C 1929	♂	153	− 6	− 0·6	0·9	2·9	13	1·3±0·3	3·2±0·2
,,	♀	161	− 7	− 0·2	0·8	3·1	14	1·6±0·3	3·3±0·2
C 1930	♂	163	− 5	0·0	1·3	3·4	12‡	1·9±0·2	3·0±0·2
,,	♀	171	− 5	− 0·3	1·4	3·6	11	1·7±0·2	3·1±0·2
L 1929	♂	100	− 7	− 1·2	1·1	7·5	17†	2·7±0·6	6·0±0·4
,,	♀	101	− 8	− 1·1	0·7	7·9	22	3·2±0·7	6·6±0·5
L 1930	♂	106	− 7	− 0·7	0·9	3·7	11††	1·7±0·4	4·1±0·3
,,	♀	107	− 8	− 0·2	1·4	4·9	12*	2·7±0·5	5·0±0·3

† 1 observation 25 gm.
‡ 1 ,, 16 gm.
†† 1 ,, 22 gm.; 1 observation 17 gm.
* 1 ,, each 19, 18, and 16 gm.

* In all subsequent references, whether in tables or text, the error of the standard deviation and other statistical characteristics cited is the standard error, which it will be remembered is greater than the probable error of the determination.

weight losses reaching 5–8 gm. occur in 25 per cent of cases, while in 50 per cent of cases weight increases never exceed 1·4 gm., and maximum increases may be as low as 0·7 gm. These latter cases clearly include the vast majority of maintained individuals, freely-growing individuals forming the bulk of the upper 50 per cent of each population. In this respect the C. and L. groups are fairly uniform whereas the upper quartiles are generally higher in the L. groups, which indicates at once a higher degree of growth in approximately half the total number of freely-growing individuals. This feature is expecially well marked during 1929. As will now be expected, mean growth is much greater among the L. groups, ranging from 1·7–3·2 gm. as against a range of 1·3–1·9 gm. among the C. groups. Dispersion in the upper parts of the populations is also greater, medians and upper quartiles ranging from 3·7–25 gm. in the L. groups as against a range of 2·9–16 gm. in the C. groups. Differences of degree of dispersion are best shown with respect to standard deviations, however, which vary between 4·1 and 6·6 gm. in the L. populations and between 3·0 and 3·3 gm. in the C. populations, with insignificant standard errors in each case. Clearly, degree of growth is distinctly different in the two sets of populations.

IV. Food Data.

The principal features of the distribution of food quantities are shown in Table 3. The median is clearly much lower in the case of the L. populations, excepting L. 1930 ♀. The figures show that 50 per cent of the first

TABLE 3.

Food Data

Population.	Sex.	No. of Fish.	Distribution of Food Quantities (= % ages of Bd.-wt.) Lower Limit.	Lower Quartile Q1.	Median Q2.	Upper Quartile Q3.	Upper Limit.	Mean Ration % $\left(\bar{y} \pm \frac{\sigma_y}{\sqrt{n}}\right)$	Standard Deviation $\left(\pm \sigma_y \pm \frac{\sigma_y}{\sqrt{2n}}\right)$
C 1929	♂	153	1	23·3	41·9	118·6	266	73·7±5·1	63·0±3·6
,,	♀	161	2	22·6	38·4	96·0	249	59·9±3·9	49·2±2·7
C 1930	♂	163	9	21·3	45·4	81·6	200	56·1±3·2	41·1±2·3
,,	♀	171	3	18·5	41·7	80·5	257	58·7±4·0	52·4±2·8
L 1929	♂	100	2	15·1	25·0	102·5	249	56·2±5·3	53·4±3·8
,,	♀	101	3	14·6	24·7	92·5	226	53·1±5·4	53·7±3·8
L 1930	♂	106	6	18·3	29·2	63·4	120	40·2±2·5	25·7±1·8
,,	♀	107	2	18·8	42·3	65·9	116	44·4±2·6	27·0±1·8

three L. populations took percentages less than 29·3, 50 per cent of any C. population taking percentages greater than 38·3. Since the portion of each population receiving such low percentages is largely if not entirely formed of maintained individuals, it follows that the maintenance demands (expressed as percentages of body-weight) at Lympstone were smaller than those at Cawsand. Weight decreases at Lympstone were slightly

greater than at Cawsand, but apparently not correspondingly so, while temperatures were slightly higher at the former place (Dawes, **1** and **2**). But in view of the fact that sizes were much greater at Lympstone it seems safe to infer that such differences in maintenance demands as are noted above are connected with, if not entirely due to, size differences. The inference also receives some support from the much smaller lower quartiles in the L. populations.

Upper quartiles and upper limits are greater in C. populations of a particular year than in L. populations of the corresponding year, especially so during 1930. Standard deviations are considerably greater in the 1929 groups and are generally greater in the C. groups when particular years are considered. The especially low standard deviations of the L. 1930 populations are connected with low growth characteristics, both degree of growth and appetite being smaller during 1930 than during the preceding year.

V. Correlation between Food and Growth.

It is evident from the foregoing that both growth in weight and food percentages taken are highly variable. This was clearly indicated also in my previous papers, where it was also shown that similar high variability characterises growth efficiency, i.e. the capacity to utilise food for purposes of growth. But high values of weight increase are generally associated with high food percentages and it is with this association that we are now concerned. Tables 8–15 are built up by noting the frequency with which particular associations of the x and y variables occur, which frequencies are grouped and arranged in arrays to form correlation tables.

The index of correlation is the correlation coefficient r, which is a pure number lying between the limits -1 and $+1$. It is positive if large values of x are associated with large values of y, negative if small values of x are associated with large values of y, and conversely. If the two variables are independent, i.e. if there is no correlation, $r=0$. It is calculated from the formula,

$$r=\frac{p}{\sigma_x\ \sigma_y},$$

where σ_x and σ_y are the standard deviations of x and y variables and where p is a measure of variance, being the product of deviations from the arbitrary origin minus the product of the difference between arbitrary and true means, i.e.

$$p=\frac{\Sigma(x-\bar{x})\,(y-\bar{y})}{n}-(D_x\,D_y)$$

Taking the C. 1929 populations and making use of the tables cited, we have :—

(Table 8) [♂]

$$D_x=\frac{51}{153}=\cdot 3 \text{ gm.}$$

$$D_y=\frac{-20}{153}=-\cdot 1307\times 10\%$$

(Table 9) [♀]

$$D_x=\frac{97}{161}=\cdot 6030 \text{ gm.}$$

$$D_y=\frac{-82}{161}=-\cdot 5093\times 10\%$$

(Table 2)

$\sigma_x=3\cdot 178$ (calculated value)

$\sigma_x=3\cdot 354$ gm.

(Table 3)

$\sigma_y=6\cdot 302\times 10\%$

$$p=\frac{1498}{153}-(\cdot 3\times -\cdot 1307)$$

$$=9\cdot 792+\cdot 044$$

$$=9\cdot 836$$

$$r=\frac{9\cdot 836}{3\cdot 178\times 6\cdot 302}=\cdot 4912$$

$\sigma_y=4\cdot 916\times 10\%$

$$p=\frac{1264}{161}-(\cdot 603\times -\cdot 5093)$$

$$=7\cdot 852+\cdot 307$$

$$=8\cdot 159$$

$$r=\frac{8\cdot 159}{3\cdot 354\times 4\cdot 916}=\cdot 4950$$

Data for the remaining populations were treated in the same way, using Tables 2 and 3 and 10–15, and the list of correlation coefficients presented in Table 4 was obtained by the use of the values of p shown. It is seen that in every case there occurs a moderately high degree of correlation. The standard errors are greater in the case of C. populations but are nowhere of sufficiently high value to preclude the probability of fairly high correlation not due to mere chance. The values of r are highest among L. populations, lowest among the C. populations, higher during 1930 in C. groups, during 1929 in L. groups. There is close similarity for the sexes during any particular year.

The diagrams in Figs. 1 and 2 are made by representing the frequencies each by a single ring in its appropriate position. They refer to the C. 1929 populations (♂ and ♀) and they indicate the degree of correlation diagrammatically. The regression lines shown are drawn in after calculation of the regression coefficients from the formulæ

$$b_x=\frac{r\,\sigma_x}{\sigma_y\times 10};\qquad b_y=\frac{r\,\sigma_y\times 10}{\sigma_x}$$

The lines cut each other in the means $\bar{x}$ and $\bar{y}$ and the angle they make with each other indicates the degree of correlation ; an angle of approximately 90° would represent independency of the variables.

Continuing the examples previously taken (C. 1929), we have :—

[♂]	[♀]
$b_x = \frac{\cdot 4912 \times 3 \cdot 178}{63 \cdot 02} = \cdot 0248$	$b_x = \frac{\cdot 495 \times 3 \cdot 354}{49 \cdot 16} = \cdot 0338$
$b_y = \frac{\cdot 4912 \times 63 \cdot 02}{3 \cdot 178} = 9 \cdot 738$	$b_y = \frac{\cdot 495 \times 49 \cdot 16}{3 \cdot 354} = 7 \cdot 256$

b_x is the regression of x on y, i.e. the regression function given by the

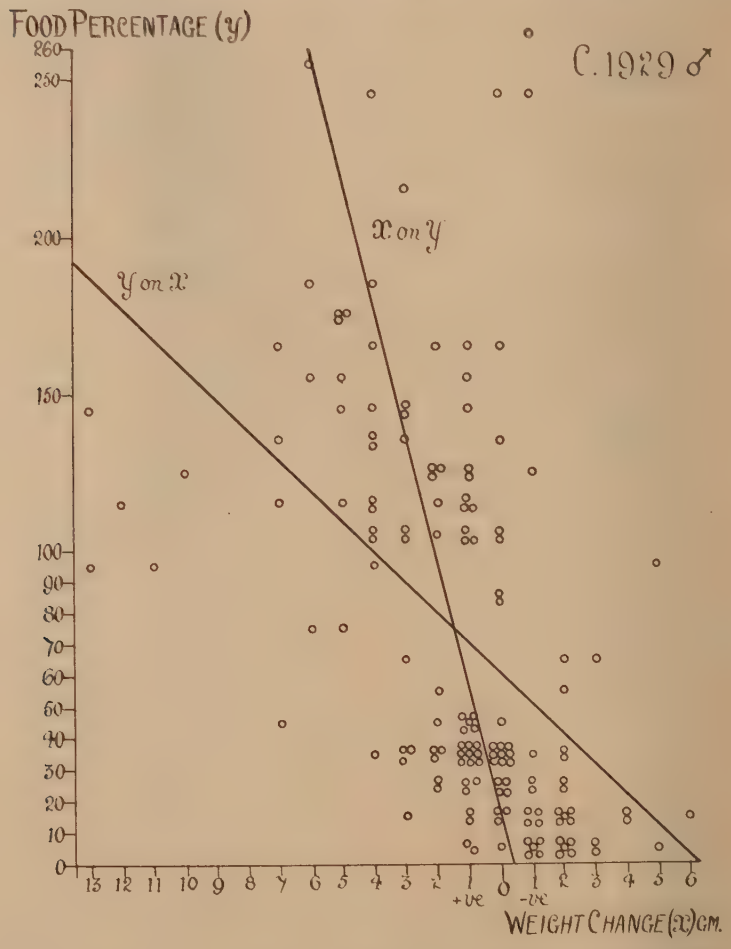

FIG. 1.—Correlation diagram for the male plaice population of Cawsand, 1929. The regression lines were drawn in lastly and are based upon the regression coefficients calculated. The diagram is a graphical presentation of Table 8.

equation $x=\bar{x}-b_x(y-\bar{y})$, by the regression of y on x, i.e. the regression function given by the equation $y-\bar{y}-b_y(x-\bar{x})$. Since the primary aim is to deduce weight increase (x) from available food percentage (y), the more important regression line from the point of view of this work is that

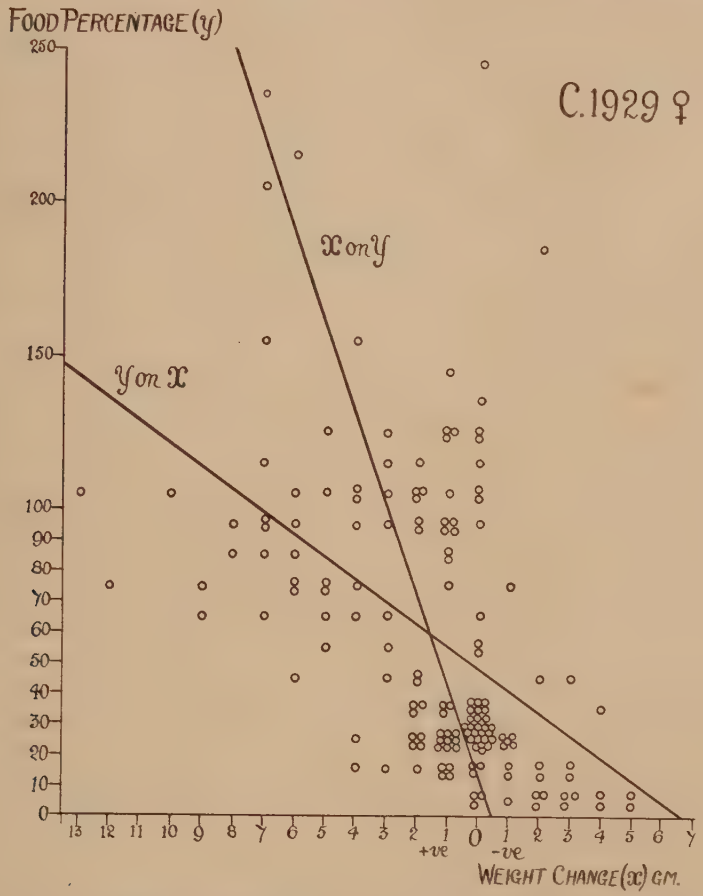

FIG. 2.—Correlation diagram for the female plaice population of Cawsand, 1929, with regression lines drawn in as in Fig. 1. The diagram is a graphical presentation of Table 9.

of x on y. The regression coefficient b_x indicates the order of change in x, i.e. weight increase or decrease, resulting on the average from unit change in y, i.e. food percentage, since $x=b_xy$. Similarly, b_y indicates the order of change in y with unit change in x. In the above example the slope of the regression line of x on y is appreciably steeper in the case of males

where b_x is smaller. Similarly, the slope of the regression line of y on x is correspondingly steeper, since the correlation coefficients are identical. This is shown diagrammatically in Figs. 1 and 2, where the regression lines are drawn into the correlation dot diagrams.

The regression coefficients for all populations were calculated by the method illustrated in the above example and they are shown in Table 4 along with corresponding standard errors. It is seen that only in the instance of the C. 1929 populations are the differences between the regression coefficients of the sexes significant, when standard errors are taken into account. Even in this instance some of this significance is lost when it is considered that the chances of the true values lying within $\pm 2\times$ standard error are approximately merely of the order of 20 to 1, and that the application of this correction in one instance renders values of the regression coefficients confluent. In all other cases it

TABLE 4.

Regression and Correlation Coefficients.

Population.	Sex.	Product of Deviations from Mean (p).	Correlation Coeff. $r=\frac{p}{\sigma_x\,\sigma_y}\pm\frac{1-r^2}{\sqrt{n}}$	Regression Coeff. $b_x=\frac{r\sigma_x}{\sigma_y}\pm\frac{\sigma_x\sqrt{1-r^2}}{\sigma_y\sqrt{n}}$	Regression Coeff. $b_y=\frac{r\sigma_y}{\sigma_x}\pm\frac{\sigma_y\sqrt{1-r^2}}{\sigma_x\sqrt{n}}$
C 1929	♂	9·84	0·49 ± ·06	·025 ± ·003	9·7 ± 1·4
,,	♀	8·16	0·49 ± ·06	·034 ± ·005	7·3 ± 1·0
C 1930	♂	7·89	0·63 ± ·03	·047 ± ·005	8·5 ± 0·9
,,	♀	10·35	0·64 ± ·03	·038 ± ·004	10·8 ± 1·0
L 1929	♂	25·09	0·78 ± ·04	·088 ± ·007	6·9 ± 0·6
,,	♀	24·97	0·70 ± ·05	·086 ± ·009	5·8 ± 0·9
L 1930	♂	6·47	0·63 ± ·04	·100 ± ·012	3·9 ± 0·5
,,	♀	8·19	0·61 ± ·04	·112 ± ·014	3·3 ± 0·4

is practically certain that no difference exists between the sexes as regards the regression functions during either year at either place.

But these functions vary slightly from year to year and vary considerably between the C. and L. populations, the Cawsand populations yielding much steeper regression lines than the Lympstone ones, and thus indicating a higher growth efficiency. And although variable factors such as temperature might conceivably produce such differences in the regression functions, there appear to be good grounds for believing that size differences, which are well marked between the C. and L. populations as we have seen, are the factors principally involved. That the differences in the regression functions are significant is shown in Table 4, the greatest value for b_x in any C. population being ·047, while in any L. population the least value of the corresponding coefficient is ·086, with an insignificant standard error in each case. These results are shown graphically in Figs. 3–5, where the regression lines for the remaining populations are presented.

In all the above cases it has been assumed that the regression lines are linear, an assumption which is by no means justifiable unless the approximation to linearity is measured and found to be reasonably close. Such a measure of the closeness to linearity is available in the form of the "correlation ratio" of Professor Pearson (η_{xy}). This ratio, which is the correlation ratio of x on y, "measures the approach of values of x

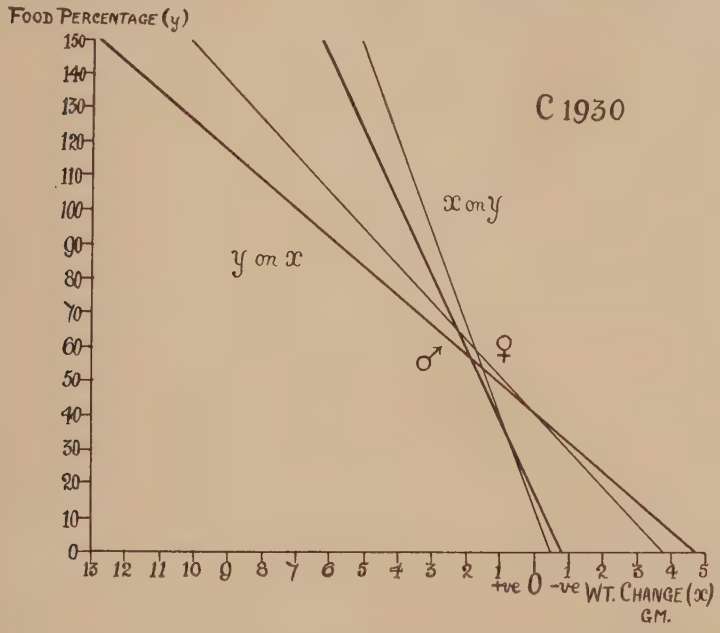

FIG. 3.—Diagram showing the positions and slopes of the regression lines for male and female plaice populations of Cawsand, 1930. Although this is not a correlation diagram, yet it is strictly based upon Tables 10 and 11.

associated with given values of y to a single valued relationship of any form" (Yule, **4**, p. 205). Each table provides two such ratios, the second being η_{yx} or the correlation ratio of y on x, the significance of which will be clear.

The correlation ratios for the various populations were calculated from the formulæ :—

$$\eta_{xy}=\frac{\sigma_{mx}}{\sigma_x}\ ; \qquad \eta_{yx}=\frac{\sigma_{my}}{\sigma_y}$$

where σ_x and σ_y=standard deviations of x and y respectively,
σ_{mx} and σ_{my} = standard deviation of the means of the x and y arrays, respectively.

The way in which the formulæ are derived is shown in *loc. cit.* and it is

sufficient here merely to indicate that η_{xy} and η_{yx} are invariably greater than the correlation coefficient r, and that the difference between these quantities indicates the departure from linearity, $\eta^2 - r^2$ measuring the divergence of the actual line through the means of the arrays from the line of regression. At the same time, it should be observed that " owing to

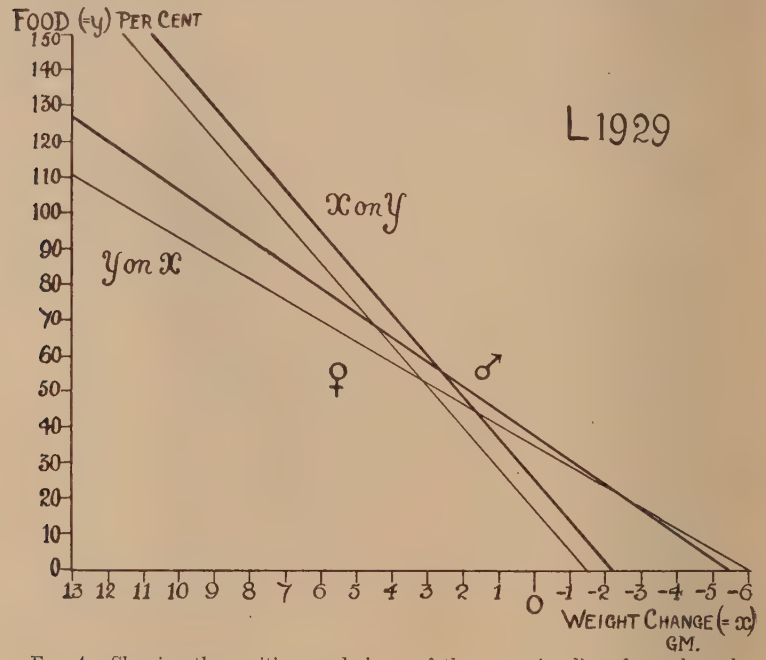

FIG. 4.—Showing the positions and slopes of the regression lines for male and female plaice populations of Lympstone, 1929, based upon Tables 12 and 13.

fluctuations of sampling, r and η are almost certain to differ slightly, even though the regression may be truly linear (Yule, **4**, p. 206).

TABLE 5.

DATA FOR ESTIMATING THE DEPARTURE OF THE REGRESSION LINES FROM LINEARITY.

Population.		Standard Deviations of Means of Arrays. σ_{mx}	σ_{my}	Standard Deviations (gm.). σ_x	σ_y	Correlation Ratios. η_{xy}	η_{yx}	Correlation Coeff. r	$\eta_{xy}^2 - r^2$	$\eta_{yx}^2 - r^2$
C 1929	♂ on ♀	2·2	37·5	3·2	63·0	·70	·60	·49	·25	·12
,,	♀ on ♂	2·3	27·1	3·3	49·2	·71	·55	·49	·26	·06
C 1930	♂ on ♀	2·1	30·1	3·0	40·1	·70	·73	·63	·09	·14
,,	♀ on ♂	2·4	36·7	3·1	52·4	·76	·70	·64	·19	·08
L 1929	♂ on ♀	5·1	49·6	6·0	53·4	·84	·93	·78	·10	·26
,,	♀ on ♂	5·6	48·5	6·6	53·7	·86	·90	·70	·25	·32
L 1930	♂ on ♀	2·7	18·9	4·1	25·7	·67	·74	·63	·05	·15
,,	♀ on ♂	3·4	19·7	5·0	27·0	·67	·73	·61	·08	·16

Values have been obtained for the correlation ratios of all populations and are shown in Table 5, together with the standard deviations from which they were calculated. If these values are compared with those of the regression coefficients, which are also shown in the table, it will be seen that in some cases the departure from linearity is fairly slight. In 4 instances, $\eta_{xy}^2 - r^2 \leqq \cdot 10$ although in other instances it increases to ·26 ; in 2 instances, $\eta_{yx}^2 - r^2 \leqq \cdot 08$ while in others it ranges from ·12 to ·32. During 1930 the approach to linearity of the regression lines is much closer than during 1929, but if it is justifiable to assume linearity in one

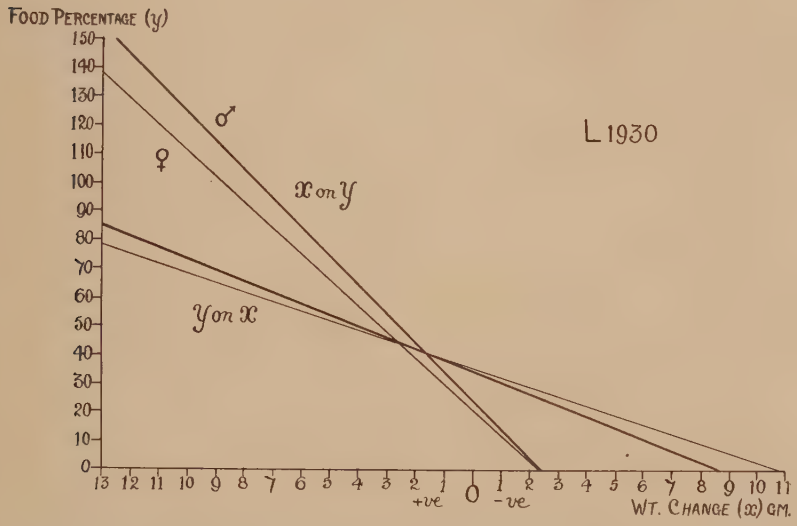

FIG. 5.—Showing the positions and slopes of the regression lines for male and female plaice populations of Lympstone, 1930. Based upon Tables 14 and 15.

instance it is justifiable in others, especially since the aim of the work is to obtain only approximate indices, and although the regression lines strictly are not straight it is sufficient for our purpose to assume that they are.

By substituting mean values and regression coefficients in the regression equations, equations of the form $x = b_x y + \text{const.}$ and $y = b_y x + \text{const.}$ are obtained. A set of pairs of such equations are provided in Table 6. The constants in equations (1) indicate how far the regression lines of x on y are displaced to the right in Figures 1–5, and it is seen that the C. and L. populations show striking differences in this respect, the constant being at least thrice the C. value in the corresponding L. population. In equations (2) the constants show how far the regression line of y on x is

displaced vertically in the same figures, and in this case there is no well-marked difference other than that shown by the C. 1929 populations, especially the male one.

TABLE 6.

REGRESSION EQUATIONS OF THE FORM $x=b_xy+$const. AND $y=b_yx+$ const., WITH VALUES OF EACH VARIATE WHEN THE VALUE OF THE OTHER IS ZERO.

Population.	Sex.	Equation (1) x=(gm.)	Equation (2) y=(%)	In (1), when x=0 y=(%)	In (2), when y=0 x=(gm.)	In (1), when x=·05 y=(%)	In (1), when x=1·0 y=(%)
C 1929	♂	·025 Y − ·49	9·7 X+60·7	19	− 6	40	60
,,	♀	·034 Y − ·42	7·3 X+48·3	12	− 7	27	42
C 1930	♂	·047 Y − ·73	8·5 X+40·0	16	− 5	26	37
,,	♀	·038 Y − ·47	10·8 X+39·8	12	− 4	26	39
L 1929	♂	·088 Y − 2·19	6·9 X+37·1	25	− 5	31	36
,,	♀	·086 Y − 1·41	5·8 X+34·8	16	− 6	22	28
L 1930	♂	·100 Y − 2·34	3·9 X+33·6	23	− 9	28	33
,,	♀	·112 Y − 2·31	3·3 X+35·5	21	− 11	25	30

If in equations (1) we make x—0, the evaluation of y will then provide a measure of maintenance requirements in terms of percentage of the initial body-weight. A list of such evaluations of y are shown in Col. 5 of Table 6, and are seen to provide greater indices in males than in females and at Lympstone than at Cawsand. This latter is contrary to previous findings both in my previous paper and in the present one where quartiles of the y variate are discussed, and it calls for an explanation.

The figures in Col. 5 of Table 6 are misleading unless it is borne in mind that there is a significant difference in the steepness of the regression lines of the C. and L. populations. These lines are much steeper in the C. populations, which implies that the change in y corresponding to unit change in x is greater in this case. When x=0, y is greater in the L. than in the C. populations, i.e. maintenance demands, as percentages, are greater. But when x=·5 the significance of the difference is lost, and when x=1·0 the values of y are considerably greater in the C. populations, as is shown in Table 6, especially during 1929. For pure maintenance, the larger L. individuals appear to require slightly higher food percentages than do the C. individuals, but this result is more apparent than real, since if very small increases are considered the reverse is the case. Increases greater than ·5 gm. required greater food percentages in the case of C. than in the case of L. populations, as is shown by a study of the regression equations.

The above-mentioned feature of relative efficiency in the C. and L. populations is brought out rather better in Table 7. The ratio $b_xX^{100}/\bar{X}$

TABLE 7.

REGRESSION COEFFICENTS INFERRED FOR STANDARD PLAICE OF 100 (b_x) AND 1 GM. (b_y) RESPECTIVELY.

Population.	C 1929 ♂	C 1929 ♀	C 1930 ♂	C 1930 ♀	L 1929 ♂	L 1929 ♀	L 1930 ♂	L 1930 ♀
$\bar{X}$	35·2	44·6	52·7	54·1	84·0	90·4	86·7	86·1
$b_x \times 100/\bar{X}$	·07	·08	·09	·07	·10	·10	·12	·13
$b_y/\bar{X}$	·28	·16	·16	·20	·08	·06	·04	·04

indicates the degree of change in x related to unit change in y for a standard plaice of 100 gm., which change is slightly greater in the L. groups, thus suggesting greater efficiency in these groups. This suggestion is furthered when the ratio $b_y/\bar{X}$ is considered, when the degree of change in y per gm. of fish corresponding to unit change in x is found to be very much greater in the C. groups, i.e. efficiency is not as great.

By making $y=0$ in equation (2) (Table 6) the values of x obtained indicate the probable weight decreases during starvation for the various populations, these being slightly greater in the L. populations (5–11 gm.) than in the C. populations (4–7 gm.).

VI. L.W. EXPERIMENTS.

It remains to consider briefly the winter experiments carried out at Lympstone during the early months of 1930. They are of especial interest because the individuals used were carried through from the experiments of 1929, which enables the results to bear comparison with those of the L. 1929 populations, thus affording also a comparison between winter and summer performances. The sexes have been grouped together, since the number of individuals of one sex is too small to allow of reliable statistical treatment.

The following size data indicate how closely the L.W. population compares with the L. 1929 ones (see Table 1) :

[$\bar{X}$] Mean weight :— $90{\cdot}6 \pm 2{\cdot}9$ gm.
[σ_x] Standard deviation :— $24{\cdot}5 \pm 2{\cdot}1$ gm.

The principal differences between the populations as regards size of individuals is thus seen to be one of degree of dispersion, this naturally being much smaller in the L.W. populations.

As would be anticipated, growth is much more restricted, as the following figures indicate :

($\bar{x}$) Mean growth :— $0{\cdot}6 \pm 0{\cdot}3$ gm.
(σ_x) Standard deviation :— $2{\cdot}6 \pm 0{\cdot}2$ gm.

Mean growth in the case of the L. 1929 populations is roughly five times as great, standard deviation more than twice as great (Table 2).

Food percentages taken are very substantially smaller.

($\bar{y}$) Mean food :— $18{\cdot}7 \pm 1{\cdot}9$ per cent.
(σ_y) Standard deviation :—$14{\cdot}6 \pm 1{\cdot}3$ per cent.

Each of these values is approximately only one-third of the corresponding values in the case of the L. 1929 populations (cp. Table 3).

As in the case of all other populations a fairly high degree of correlation occurs between food and growth, since from Table 16,

$$p = \frac{322}{62} - (\cdot 629 \times \cdot 742)$$
$$= 4{\cdot}727$$

and

$$r = \frac{p}{\sigma_x \sigma_y} = \frac{4{\cdot}727}{2{\cdot}57 \times 2{\cdot}93} = \cdot 6276\ (\cdot 63 \pm \cdot 08).$$

The correlation coefficient is smaller, however, than those of the L. 1929 populations, which are ·78 (♂) and ·70 (♀) respectively (Table 4).

The regression coefficients of x on y, i.e. b_x, of the L. 1929 populations were seen to be distinctly uniform, the differences between the sexes being negligible (Table 4). The corresponding coefficient of the L.W. populations provides a striking contrast, for

$$b_x = \frac{\cdot 628 \times 2{\cdot}57}{2{\cdot}93 \times 5} = \cdot 110\ (\pm \cdot 017).$$

Change in x corresponding to unit change in y is thus, on the average, greater during the winter months than during the summer months, in the ratio of 110 : 87, i.e. growth efficiency is enhanced even though growth is restricted. (Note : Strictly, this relates to the onset of a new growth season, that of 1930, since the individuals showing growth are those which have passed through the winter period of growth inhibition. In this connection it is interesting to observe that the L.W. regression coefficient b_x agrees very closely with the corresponding coefficients of the L. 1930 groups.)

By substitution of mean values ($\bar{x}$ and $\bar{y}$) in the regression equation, the following equation is obtained :

$$x = \cdot 110y - 1{\cdot}43$$

By making x=0, it is found that y=13, so that, on the average, maintenance demands are reduced during the winter months in the ratio of $^{13}/_{16\text{–}25}$. If small weight increases are permitted, the reduction is more strongly marked, for when x=·5, y=17, so that the ratio becomes $^{17}/_{28\text{–}36}$.

Corresponding contrast is seen in the case of the regression of y on x, for the regression coefficient

$$b_y = \frac{\cdot 628 \times 2 \cdot 93 \times 5}{2 \cdot 57} = 3 \cdot 576 \ (3 \cdot 6 \pm \cdot 6)$$

(cp. Table 4, L. 1929). After substitution of means, the regression equation becomes

$$y = 3 \cdot 58x + 20 \cdot 8.$$

Thus the regression line of y on x is shifted nearer to the y zero co-ordinate, that of x on y being shifted nearer to the x zero co-ordinate, during the winter months. Moreover, both regression lines are considerably less steep than during the previous summer.

VII. Notation Used.

$\bar{X}$	Mean weight.
σ_x	Standard deviation of weight.
$\bar{x}$	Mean growth in weight.
σ_x	Standard deviation of growth.
$\bar{y}$	Mean percentage of initial body-weight as food.
D_x	Difference between arbitrary and true weight means.
D_y	Difference between arbitrary and true percentage means.
f	Frequency.
n	Total number of frequencies.
Σ	Summation.
r	Correlation coefficient.
b_x	Regression coefficient of x on y.
b_y	Regression coefficient of y on x.
x, y	Any value of the variates within ranges stated.
p	Product of deviations from means.
σ_{mx}	Standard deviations of means of x arrays.
σ_{my}	Standard deviations of means of y arrays.
η_{xy}	Correlation ratio of x on y.
η_{yx}	Correlation ratio of y on x.
$\eta^2 - r^2$	A measure of the divergence of the actual line through the means of arrays from line of regression.

VIII. SUMMARY.

The aim of the paper has been to treat the data of the Plaice experiments on Growth and Maintenance, carried out at Cawsand and Lympstone during 1929 and 1930, in a statistical manner. The experiments for each sex, year and place have been resolved into experiments with an

average plaice during the "average" 14 days of the third growth season. Growth and food-percentage indices have been provided and a fairly high degree of correlation between food and growth has been indicated, and shown to be uniform for the sexes. Regression coefficients indicating the facility with which food is utilised for purposes of growth have been worked out and have been shown to vary with place and year, but not with sex. Maintenance demands have been evaluated and shown to be slightly greater in males than in females. The diminished demands during winter months have also been evaluated. The outstanding features of the results are the distinct differences shown between the Cawsand and Lympstone plaice, which differences it is suggested arise out of size differences to a large extent.

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TABLE 8.

Correlation Table of Percentage of Body-Wt. as food and Wt. Increase. Cawsand Males [1929].

Percentage of Body-wt. as Food (=y).	Wt. Increase (gm.) (=x).												+ve		−ve								
	13	12	11	10	9	8	7	6	5	4	3	2	1	0	1	2	3	4	5	6	Total	f×Dy	f×D²y
265															1						1	19	361
255								1													1	18	324
245										1				1	1						3	51	867
235																					0	0	0
225																					0	0	0
215											1										1	14	196
205																					0	0	0
195																					0	0	0
185								1		1											2	22	242
175									3												3	30	300
165							1			1		1	1	1							5	45	405
155								1	1				1								3	24	192
145	1								1	1	2		1								6	42	294
135							1			2	1			1							5	30	180
125				1								3	2		1						7	35	175
115		1					1		1	2		1	3								9	36	144
105										2	2	1	3	2							10	30	90
95	1		1							1					1				1		5	10	20
85														2							2	2	2
75								1	1												2	0	0
65											1					1	1				3	−3	3
55												1				1					2	−4	8
45							1					1	5	1							8	−24	72
35										1	3	3	9	9	1	2					28	−112	448
25												2	3	4	2	2					13	−65	325
15											1		2	3	4	5		2		1	18	−108	648
5													2	1	5	5	2		1		16	−112	784
Total	2	1	1	1	0	0	4	4	7	12	11	13	32	25	16	16	3	2	2	1	153	−20	6080
f×Dx	24	11	10	9	0	0	24	20	28	36	22	13	0	−25	−32	−48	−12	−10	−12	−7	+51		
f×D²x	288	121	100	81	0	0	144	100	112	108	44	13	0	25	64	144	48	50	72	49	1563		
np(x−x̄) (y−ȳ)	108	44	20	45	0	0	96	185	196	204	42	4	0	44	60	258	60	60	30	42	1498		

TABLE 9.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Cawsand Females [1929].

Percentage of Body-wt. as Food (= y).	Wt. Increase (gm.) (= x).																							
	14	13	12	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	6	7	Total	f×Dy	f×D²y
245															1								1	18	324
235																							1	17	289
225																							0	0	0
215																							1	15	225
205																							1	14	196
195																							0	0	0
185																	1						1	12	144
175																							0	0	0
165																							0	0	0
155								1															2	18	162
145														1									1	8	64
135	1													1	1								3	21	147
125												1		3	2								7	42	252
115								1				1	1		1								4	20	100
105		1			1				1	1	2	1	3	1	2								13	52	208
95							1	2	1		1	1	2	4	1								13	39	117
85							1	1	1					2									5	10	20
75			1			1			2	2	1			1		1							9	9	9
65						1		1		1	1	1			1								6	0	0
55										1		1			2								4	−4	4
45									1			1	2				1	1					6	−12	24
35													3	3	9				1				16	48	144
25											1		4	9	17	5							36	144	576
15											1	1	1	4	3	2	2	2				1	17	−85	425
5															3	1	3	3	2	2			14	84	504
Total	1	1	1	0	1	2	2	8	7	6	8	8	16	29	43	9	7	6	3	2	0	1	161	−82	3934
f×Dx	13	12	11	0	9	16	14	48	35	24	24	16	16	0	48	−18	−21	−24	−15	−12	0	−8	−97		
f×D²x	169	144	121	0	81	128	98	288	175	96	72	32	16	0	43	36	63	96	75	72	0	64	1869		
np(x−x̄) (y−ȳ)	91	48	11	0	36	8	35	318	120	44	36	20	−11	0	77	70	54	120	75	72	0	40	1264		

TABLE 10.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Cawsand Males [1930].

Percentage of Body-wt. as Food (=y).	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	Total	f×Dy	f×D²y
205														1									1	16	256
165													1										1	12	144
155									1				1										2	22	242
145					1					1													2	20	200
135									1	1		1	2			1							6	54	486
125												1	1	1									3	24	192
115						1									1	3							5	35	245
105						1	1		3			1	3										9	54	324
95											1		2	1							1		5	25	125
85	1												2		2	2	1						8	32	128
75												4		3	3								10	30	90
65												1		2	2	4	2						11	22	44
55													2	3	3	1	1	2					12	12	12
45									1		1		2	1	3	2	3			1			14	0	0
35														1		4	3	1					9	−9	9
25															5	8	10	4		1			28	−56	112
15														1	2	7	11	8	6	1			36	−108	324
5																						1	1	−4	16
Total	1	0	0	0	1	2	1	0	6	2	2	8	16	14	21	32	31	15	6	3	1	1	163	+181	2949
f×Dx	15	0	0	0	11	20	9	0	42	12	10	32	48	28	21	0	−31	−30	−18	−12	−5	−6	+146		
f×D²x	225	0	0	0	121	200	81	0	294	72	50	128	144	56	21	0	31	60	54	48	25	36	1646		
np(x−x̄)(y−ȳ)	60	0	0	0	110	130	54	0	266	114	25	148	261	82	15	0	47	62	54	20	−25	24	1447		

Wt. Increase (gm.) (=x).

TABLE 11.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Cawsand Females [1930].

Percentage of Body-wt. as Food (=y).	Wt. Increase (gm.) (=x).																			
	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	Total	f×Dy	f×D²y
255											1							1	20	400
245									1									1	19	361
235																		0	—	—
225									1									1	17	289
215																		0	—	—
205																		0	—	—
195			1							1								2	28	392
185																		0	—	—
175				1														1	12	144
165				1	1		2											4	44	484
155		2					1											3	30	300
145				2		1	1	1		1								7	63	567
135						1	1											2	16	128
125							1		1									3	21	147
115	1		1				1	1			1							5	30	180
105					1				1	1								3	15	75
95					1	2		1	1									5	20	80
85							2		1	2								5	15	45
75							1	2	2									5	10	20
65				1			1	1	1	3	2						1	10	10	10
55						1	3	1	3	3	1	3						15	0	0
45						1		1	2	4	5	1		1				15	- 15	15
35										2	3	4		1				10	- 20	40
25								1	1	3	3	5	5	2	2		1	23	- 69	207
15							1	1		4	4	16	9	7	4		1	47	−188	752
5													2			1		3	−15	75
Total	1	2	2	5	3	6	15	10	15	24	20	31	16	11	6	1	3	171	+ 63	4711
f×Dx	9	16	14	30	15	24	45	20	15	0	−20	−62	−48	−44	−30	−6	−21	−43		
f×D²x	81	128	98	180	75	96	135	40	15	0	20	124	144	176	150	36	147	1645		
np(x−x̄)(y−ȳ)	54	160	140	252	100	96	201	32	55	0	8	144	183	148	110	30	42	1755		

TABLE 12.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Lympstone Males [1929].

Percentage of Body-wt. as Food (=y).	Wt. Increase (gm.) (=x).																														
	25	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	6	7	Total	f×Dy	f×D²y
245	1																										1	19	361
175						1																					1	12	144
165									1					1													2	22	242
155										1																	1	10	100
145			1					1			2	1															5	45	405
135									1			1	1														3	24	192
125					2			1							1												4	28	196
115														1		2											5	30	180
105										1				1				2									4	20	50
95		1		1																							3	12	48
85			1	1								1	1					1									5	10	45
75																											0	0	0
65								1								1			1								3	3	3
55										1																	1	0	0
45																1			1	1							3	−3	3
35																						1					1	−2	4
25															1	1	4	2	5	2	1						16	−48	144
15												1			1	3	1	4	5	7	3	2	2	3	2	1	35	−140	560
5																		1			1	2	2		1		7	−35	175
Total	1	1	2	2	2	1	0	3	2	3	4	4	2	3	3	8	5	10	12	10	6	5	4	3	3	1	100	+12	2852
f×Dx	23	15	28	26	24	11	0	27	16	21	24	20	8	9	6	8	0	−10	−24	−30	−24	−25	−24	−21	−24	−9	+75		
f×D²x	529	225	392	338	288	121	0	243	128	147	144	100	32	27	12	8	0	10	48	90	96	125	144	147	192	81	3667		
np(x−x̄)(y−ȳ)	437	60	168	91	168	132	0	153	152	105	180	80	44	66	0	−3	0	14	70	105	64	100	108	84	104	36	2518		

TABLE 13.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Lympstone Females [1929].

Percentage of Body-wt. as Food (=y).	Wt. Increase (gm.) (=x).																													(f)		
	22	21	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	6	8	Total	f×Dy	f×D²y
225												1																	1	17	289
205										1																			1	15	225
195													1																1	14	196
185													1																1	13	169
175															1														1	12	144
165																													0	0	0
155																													1	10	100
145								1						1															2	18	162
135					1			1				1					1												4	32	256
125										1					1														2	14	98
115			1												1				1										3	18	108
105		1				1		1			1					1		1											6	30	150
95				1				1			1																		3	12	48
85	1				1					1																			3	9	27
75							1																						1	2	4
65							1				1										1					1			4	4	4
55												1									1								2	0	0
45																					2								4	−4	4
35																						1							1	−2	4
25																1		3	1	4	4	2	1		2				18	−54	162
15																			3	5	7	2	3	5	3	2		1	31	−124	496
5																					3	5			2		1		11	−55	275
Total	1	1	1	1	2	2	2	4	1	3	3	3	2	1	3	2	1	5	5	9	18	10	4	5	7	3	1	1	101	−19	2921
f×Dx	18	17	14	13	24	22	20	36	8	21	18	15	8	3	6	2	0	−5	−10	−27	−72	−50	−24	−35	−56	−27	−10	−12	−83		
f×D²x	324	289	196	169	288	242	200	324	64	147	108	75	32	9	12	2	0	5	20	81	288	250	144	245	448	243	100	144	4449		
np(x−x̄) (y−ȳ)	54	85	84	52	132	165	30	234	−8	175	60	125	108	27	50	2	0	5	18	96	224	205	99	140	224	63	50	48	2538		

TABLE 14.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Lympstone Males [1930].

Percentage of Body-wt. as Food (=y).	Wt. Increase (gm.) (=x). 22	17	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	6	7	Total	f×Dy	f×D²y
115										1												1	8	64
105			1		1																	0	0	0
95				2					1													2	12	72
85						1	1		2	1		2	1	1								3	15	75
75	1						1	1	5	1	2	2	1	1	1							10	40	160
65		1						1	1	3		1	2		1	1		1				16	48	144
55									1		1				1							10	20	40
45									1						1							3	3	3
35											1	1		1			1	1	1			6	0	0
25									1			3	5	7	5	3						24	−24	24
15										1		3	2	9	7	2	2					26	−52	104
5												2		1							2	5	−15	45
Total	1	1	1	2	1	1	2	1	11	7	4	14	11	20	15	6	3	2	1	0	2	106	+55	731
f×Dx	21	16	10	18	8	7	12	5	44	21	8	14	0	−20	−30	−18	−12	−10	−6	0	−16	+72		
f×D²x	441	256	100	162	64	49	72	25	176	63	16	14	0	20	60	54	48	50	36	0	128	1834		
np(x−x̄) (y−ȳ)	84	48	60	90	48	28	42	15	120	57	14	1	0	21	26	15	16	−10	0	0	48	723		

TABLE. 15.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Lympstone Females [1930].

Percentage of Body-wt. as Food (= y).	19	18	17	16	12	11	10	9	8	7	6	5	4	3	2	+ve 1	0	−ve 1	2	3	4	5	6	7	8	Total	f×Dy	f×D²y
115						2																				2	14	98
105																										0	0	0
95						1						1														2	10	50
85				1			1			1	1	1	1	1	1											8	32	128
75	1				2	1	1				1			1												7	21	63
65		1				1	1	2		1	1		2	3	3	1	2	1								19	38	76
55						1					1	1			1	1				1	1					7	7	7
45								1							2	2	5						1			11	0	0
35								1							2	1	1	1				1			1	8	−8	8
25															1	2	7	3								13	−26	52
15											1		1		3	4	9	4	2			1	1			26	−78	234
5													1	1		1								1		4	−16	64
Total	1	1	0	1	2	6	3	4	0	2	5	3	5	6	13	12	24	9	2	1	1	2	2	1	1	107	−6	780
f×Dx	17	16	0	14	20	54	24	28	0	10	20	9	10	6	0	−12	−48	−27	−8	−5	−6	−14	−16	−9	−10	+73		
f×D²x	289	256	0	196	200	486	192	196	0	50	80	27	20	6	0	12	96	81	32	25	36	98	128	81	100	2687		
$n_p(x-\bar{x})(y-\bar{y})$	51	32	0	56	60	225	72	21	0	30	28	30	2	9	0	18	76	51	24	−5	−6	28	24	36	10	872		

Wt. Increase (gm.) (= x).

TABLE 16.

Correlation Table of Percentage of Body-Wt. as Food and Wt. Increase. Lympstone Winter ♂ and ♀ [1930].

	Wt. Increase (gm.) (=x).																
								+ve		−ve							
	8	7	6	5	4	3	2	1	0	1	2	3	4	5	Total	f×D_y	f×D^2_y
65				1											1	10	100
60								1							1	9	81
55				1		1									2	16	128
50										1					1	7	49
45			1												1	6	36
40			1												1	5	25
35				1	1		1								3	12	48
30				1											1	3	9
25	1					2		1							4	8	16
20							3	2	2	2					9	9	9
15							2	3	2	2	2	1			12	0	0
10					1		2	2	4	2	1	1			13	−13	13
5									1	5	4	2		1	13	−26	52
Total	1	0	2	4	2	3	8	9	9	12	7	4	0	1	62	+46	566
f×Dx	8	0	12	20	8	9	16	9	0	−12	−14	−12	0	−5	+39		
f×D^2x	64	0	72	100	32	27	32	9	0	12	28	36	0	25	437		
np(x−$\bar{x}$) (y−y)	16	0	66	125	12	36	10	11	0	3	18	15	0	10	322		

Growth in Length during the Transition from Larva to Adolescent in the Pilchard and Sprat.

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With 1 Figure in the Text.

Introduction.

In a previous paper (Ford, 1) it was shown that during the transition from larva to adolescent in the herring, there was a progressive alteration in position of the anus and fins relative to one another and to the vertebrae. Simple models of tape and elastic were utilised to demonstrate how such changes in position could be induced on the assumption that different intervals along the body grew in length at unequal rates. It will be recalled that for the herring it was accepted that two such intervals, namely, (1) from the back of the head, along the dorsal surface, to the first ray of the dorsal fin; and (2) from the insertion of the pelvics, along the ventral surface to the anus, remained at a steady unchanging length throughout the transition stages. Meanwhile, other body-intervals increased in length, each at its own rate.

With the necessary material available for the study, similar investigations have since been made for the sprat and the pilchard, and it is the purpose of the present publication to discuss the results so obtained.

Methods.

As in the case of the young herrings, determinations of the length of the following body-intervals were made (see Fig. 1):—

Head	Hd.
Back of head to first ray of dorsal fin	Hd. to D^1.
First ray of dorsal fin to base of caudal peduncle	D^1 to Caud. Ped.
Back of head to pelvics	Hd. to Pv.
Pelvics to anus	Pv. to An.
Anus to base of caudal peduncle	An. to Caud. Ped.
Snout to base of caudal peduncle	L_B.

Having completed the measurement, the data were grouped into classes according to the length of (L_B–Hd.), that is to say, according to the length of the distance from the back of the brain to the base of the caudal peduncle.

The Pilchard (*Clupea pilchardus* Walbaum).

The data for a total of 261 measured specimens were as shown in Table 1. (See p. 979.) Examining the figures relating to the two intervals (Hd. to D^1) and (Pv. to An.), it is seen that (Hd. to D^1) varies but little from an average figure of about 10·5 mm., whereas the values of (Pv. to An.) tend to increase as the value of (L_B–Hd.) increases. The pilchard thus resembles the herring in that the length from the back of the brain to the first dorsal ray appears to remain steady throughout the transition from larva to adolescent, but differs from the herring in that the distance from the pelvics to the anus increases instead of remaining unchanged throughout metamorphosis.

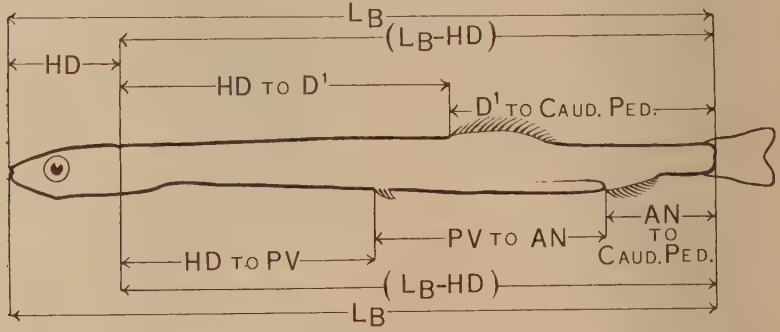

Fig. 1.—Diagrammatic representation of young clupeoid to illustrate meaning of symbols used in the text.

Snout to end of caudal peduncle	L_B.
Snout to back of head	Hd.
Back of head to end of caudal peduncle	(L_B–Hd.)
Back of head to first ray of dorsal fin	Hd. to D^1.
First dorsal ray to end of caudal peduncle	D^1 to Caud. Ped.
Back of head to pelvics	Hd. to Pv.
Pelvics to anus	Pv. to An.
Anus to end of caudal peduncle	An. to Caud. Ped.

With regard to the data in general, it has been determined that the length of a given body-interval can be expressed in terms of (L_B–Hd.) in accordance with a simple equation of the form :

$$Y=M(X)+C$$

TABLE 1.

AVERAGE VALUES (MM.) FOR EACH OF THE FOLLOWING VALUES OF L_B–HD :—

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Hd. . .	3·5	3·8	3·7	4·2	4·9	5·0	—	6·2	6·3	6·3	6·7	7·0	7·1	7·6	7·6	8·6	9·3	9·5	9·5
Hd. to D^1 .	10·0	9·8	10·5	10·5	10·6	10·8	—	10·2	10·2	10·2	10·2	10·1	10·4	10·3	10·8	10·4	10·8	10·7	12·0
D^1 to Caud. Ped. .	8·0	9·2	9·5	10·5	11·4	12·2	—	14·8	15·8	16·8	17·8	18·9	19·6	20·7	21·2	22·6	23·2	24·3	24·0
Hd. to Pv. .	6·4	6·8	7·3	7·9	8·4	8·7	—	9·75	10·2	10·4	10·8	11·0	11·5	11·8	12·4	12·7	13·6	14·5	15·0
Pv. to An. .	7·8	8·0	8·3	8·2	8·2	8·3	—	8·0	8·0	8·4	8·4	8·8	9·0	9·2	9·2	9·6	8·8	9·3	9·0
An. to Caud. Ped. .	3·6	4·2	4·4	4·9	5·4	6·0	—	7·25	7·8	8·2	8·8	9·2	9·5	10·0	10·4	10·7	11·6	11·2	12·0
Total No. of Spec. .	5	9	19	15	5	6	—	12	30	39	26	22	21	17	17	9	5	3	1

TABLE 2.

VALUES CALCULATED FROM EQUATIONS—VALUES OF (L_B–HD).

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Hd. to D^1 .	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5	10·5
D^1 to Caud. Ped. .	7·5	8·5	9·5	10·5	11·5	12·5	13·5	14·5	15·5	16·5	17·5	18·5	19·5	20·5	21·5	22·5	23·5	24·5	25·5
Hd. to Pv. .	6·43	6·87	7·31	7·75	8·19	8·63	9·06	9·50	9·94	10·37	10·81	11·25	11·68	12·12	12·56	13·00	13·44	13·87	14·31
Pv. to An. .	8·07	8·13	8·19	8·25	8·31	8·37	8·44	8·50	8·56	8·63	8·69	8·75	8·82	8·88	8·94	9·00	9·06	9·13	9·19
An. to Caud. Ped. .	3.5	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	12·0	12·5

The following equations have been found to give values which approximate reasonably closely to the observed figures :—

(1) (Hd. to D^1) $=10{\cdot}5$.
(2) (D^1 to Caud. Ped.) $=(L_B\text{–Hd.})-10{\cdot}5$.
(3) (Hd. to Pv.) $={\cdot}4375\ (L_B\text{–Hd.})-1{\cdot}44$.
(4) (Pv. to An.) $={\cdot}0625\ (L_B\text{–Hd.})+6{\cdot}94$.
(5) (An. to Caud. Ped.) $=(L_B\text{–Hd.})-(\text{Hd. to Pv.})-(\text{Pv. to An.})$.

How nearly the calculated values approximate to the raw data may be learned by comparing Table 2 with Table 1. (See p. 979.) Accepting equations 1 to 5 above, it is possible to make the following statements :—

For each unit of increase in (L_B–Hd.).

(1) Hd. to D^1 increases by 0·0000 unit.
(2) D^1 to Caud. Ped. ,, ,, 1·0000 ,,
(3) Hd. to Pv. ,, ,, 0·4375 ,,
(4) Pv. to An. ,, ,, 0·0625 ,,
(5) An. to Caud. Ped. ,, ,, 0·5000 ,,

The Sprat (*Clupea sprattus* L.).

The corresponding data for a total of 136 sprats were :—

TABLE 3.

Average Values (mm.) for each of the following Values of (L_B–Hd.).

	18	19	20	21	22	23	24	25	26
Hd.	3·75	3·7	3·9	4·0	4·3	4·7	4·9	5·1	5·5
Hd. to D^1	9·25	9·6	10·0	10·7	10·7	11·1	11·7	11·9	11·8
D^1 to Caud. Ped.	8·75	9·4	10·0	10·3	11·3	11·9	12·3	13·1	14·2
Hd. to Pv.	7·1	7·5	8·1	8·8	9·2	9·7	10·7	11·5	11·5
Pv. to An.	6·4	6·4	6·7	6·4	6·5	6·2	5·6	6·5	5·7
An. to Caud. Ped.	4·5	5·1	5·2	5·8	6·3	7·1	7·7	7·0	8·8
Total No. of Spec.	8	19	15	19	25	16	14	14	6

Referring as before to the values of (Hd. to D^1) and (Pv. to An.), it is seen that the former interval increases as the value of (L_B–Hd.) increases, while the latter oscillates about a mean of 6·3, with perhaps a tendency even to decrease as (L_B–Hd.) increases. Thus the sprat agrees with the herring in that the distance from the pelvics to the anus does not increase during the transition from larva to adolescent, but differs from it in that the distance from the back of the brain to the first dorsal ray

increases instead of remaining at a steady length. Comparing the sprat with the pilchard, it is seen that the interval (Hd. to D^1) increases in the sprat but remains steady in the pilchard, whereas the interval (Pv. to An.) remains steady in the sprat but increases in the pilchard. These comparisons may be summarised thus :—

	HERRING.	PILCHARD.	SPRAT.
Hd. to D^1 . .	Constant.	Constant.	Increases with L_B.
Pv. to An. . .	Constant.	Increases with L_B.	Constant.

Utilising the data in general, it has been found that the following equations may be used as mathematical summaries for the sprat :—

(1) (Hd. to D^1) $=\cdot412\ (L_B\text{–Hd.})+1\cdot79$.
(2) (D^1 to Caud. Ped.) $=(L_B\text{–Hd.})-(\text{Hd. to } D^1)$.
(3) (Hd. to Pv.) $=\cdot571\ (L_B\text{–Hd.})-3\cdot2$.
(4) (Pv. to An.) $=6\cdot26$.
(5) (An. to Caud. Ped.) $=(L_B\text{–Hd.})-(\text{Hd. to Pv.})-(\text{Pv. to An.})$.

In Table 4, the values calculated from the above equations are shown for values of (L_B–Hd.), ranging from 18 mm. to 26 mm. As was the case with the pilchard, it is seen that the calculated values (given in Table 4) are not greatly different from the observed data (given in Table 3).

TABLE 4.

VALUES OF (L_B–HD.).

	18	19	20	21	22	23	24	25	26
Hd. to D^1 . . .	9·2	9·61	10·02	10·44	10·85	11·26	11·67	12·09	12·50
D^1 to Caud. Ped. . .	8·8	9·39	9·98	10·56	11·15	11·74	12·33	12·91	13·50
Hd. to Pv. . . .	7·09	7·66	8·23	8·8	9·37	9·94	10·52	11·09	11·66
Pv. to An. . . .	6·26	6·26	6·26	6·26	6·26	6·26	6·26	6·26	6·26
An. to Caud. Ped. . .	4·65	5·08	5·51	5·94	6·37	6·80	7·22	7·65	8·08

Thus for each unit of increase in the length of (L_B–Hd.) :

(1) Hd. to D^1) increases by ·412 unit.
(2) D^1 to Caud. Ped. ,, ,, ·588 ,,
(3) Hd. to Pv. ,, ,, ·571 ,,
(4) Pv. to An. ,, ,, ·000 ,,
(5) An. to Caud. Ped. ,, ,, ·429 ,,

COMPARISONS BETWEEN SPECIES.

Consider, first, the growth in length along the dorsal surface. An increase of one unit in the length of (L_B–Hd.) is in each species the sum

of the increases of (Hd. to D^1) and (D^1 to Caud. Ped.). These latter are as follows :—

INCREASES PER UNIT INCREASE IN (L_B–Hd.).

	Herring.	Pilchard.	Sprat.
Hd. to D^1	nil	nil	·412
D^1 to Caud. Ped. . .	1·0	1·0	·588
Total . .	1·0	1·0	1·000

Thus for each unit increase in the length of (L_B–Hd.) the first dorsal ray is being brought relatively nearer the head in all three species, but more slowly in the sprat than in the herring or pilchard.

Unit increase in length of (L_B–Hd.) along the *ventral* surface is shared among three intervals, namely (Hd. to Pv.), (Pv. to An.), and (An. to Caud. Ped.). In the three species these individual increments are :—

INCREASES PER UNIT INCREASE IN (L_B–Hd.).

	Herring.	Pilchard.	Sprat.
(Hd. to Pv.) . . .	·455	·4375	·571
(Pv. to An.) . . .	nil	·0625	nil
(An. to Caud. Ped.) . .	·545	·5000	·429
Total . .	1·000	1·0000	1·000

It is seen that in the sprat the greater part of each unit increase in (L_B–Hd.) is added to the interval (Hd. to Pv.), whereas in both the herring and pilchard, particularly the former, the major increase is in the interval (An. to Caud. Ped.). Dropping the use of symbols, this is to say that in the sprat the greater part of each unit increase in the body-length is added in front of the pelvic fins, whereas in the herring and pilchard it is added post-anally.

It is instructive, also, to consider the changes in relative positions of the pelvic fins and anus as the result of unit increase in the length of (L_B–Hd.). Referring to Table 2, it is noted that in a pilchard in which (L_B–Hd.) is 18 mm. the interval (Hd. to Pv.) measures 6·43 mm. When (L_B–Hd.) is 19 mm. the interval (Hd. to Pv.) is 6·87 mm. That is to say, an increase of 1 mm. in (L_B–Hd.) results in an alteration of the proportion $\frac{\text{(Hd. to Pv.)}}{\text{(L_B–Hd.)}}$ from $\frac{6{\cdot}43}{18}$ to $\frac{6{\cdot}87}{19}$, or from ·357 to ·361. In effect this means that as the result of unit increase in (L_B–Hd.) the pelvics make a relative movement away from the head. Again referring to Table 2, it is noted that in a pilchard in which (L_B–Hd.) is 18 mm. the interval (An. to Caud. Ped.) is 3·5 mm., whereas at 19 mm. the same interval measures 4·0 mm. Thus an increase of 1 mm. in (L_B–Hd.) results

in an alteration of the proportion $\frac{\text{(An. to Caud. Ped.)}}{(L_B\text{–Hd.})}$ from $\frac{3{\cdot}5}{18}$ to $\frac{4{\cdot}0}{19}$, or from ·19 to ·21. This implies a relative movement of the anus towards the head. Hence in the pilchard each unit increase in (L_B–Hd.) causes a slight alteration in relative position of both pelvic fins and anus, the movement being *away* from the head in the case of the pelvics, but *towards* the head in the case of the anus.

Turning to the sprat, it is seen from Table 4 that when (L_B–Hd.) is 18 mm. the interval (Hd. to Pv.) measures 7·09 mm., while at 19 mm. it is 7·66 mm. long. For unit increase in (L_B–Hd.) therefore the proportion $\frac{\text{(Hd. to Pv.)}}{(L_B\text{–Hd.})}$ changes from $\frac{7{\cdot}09}{18}$ to $\frac{7{\cdot}66}{19}$, or from ·39 to ·40. Hence, as in the pilchard, the pelvics make a relative movement away from the head, but it is here very slight. Table 4 also shows that in the sprat in which (L_B–Hd.) is 18 mm. the interval (An. to Caud. Ped.) measures 4·65 mm., while at 19 mm. it is 5·08 mm. Unit increase in (L_B–Hd.) results in an alteration of the proportion $\frac{\text{(An. to Caud. Ped.)}}{(L_B\text{–Hd.})}$ from $\frac{4{\cdot}65}{18}$ to $\frac{5{\cdot}08}{19}$, or from ·26 to ·27. Hence, as in the pilchard, there is a relative movement of the anus towards the head for each unit increase in (L_B–Hd.). Now it has already been shown (Ford, 1) that in the herring the pelvics move backward and the anus forward relatively as (L_B–Hd.) increases, so that all three species are alike in this respect.

Before finishing this discussion of growth along the ventral surface, attention is directed to the relative lengths of the ventral body-intervals as (L_B–Hd.) increases. From Table 2 it is learned that in the pilchard of 18 mm. the interval (Hd. to Pv.) is shorter than (Pv. to An.), but that with increasing length of (L_B–Hd.) the difference between these two intervals becomes less and less until at 23 mm. the interval (Hd. to Pv.) actually exceeds that of (Pv. to An.). From then onwards the margin of excess increases steadily. In the sprat, however (see Table 4), the interval (Hd. to Pv.) is already greater than (Pv. to An.) when (L_B–Hd.) is at its smallest length of 18 mm., and successive increases in (L_B–Hd.) merely augment the initial difference between the two intervals. Again, in both the pilchard and sprat the length of (Pv. to An.) at 18 mm. is greater than that of (An. to Caud. Ped.), but as the fish grows in length the difference between the intervals diminishes until both are of the same length. This occurs at ca. 28 mm. in the pilchard, but at ca. 21·5 mm. in the sprat. At greater lengths of (L_B–Hd.) the interval (An. to Caud. Ped.) is longer than (Pv. to An.).

Having considered growth in length along the dorsal surface, as distinct from that along the ventral surface, it is now desirable to discuss the one in conjunction with the other. We have seen that in the three species the dorsal fin makes a relative movement towards the head, the pelvics away from the head, and the anus towards it. How does the position of the first dorsal ray alter with respect to the pelvics and anus? Consider, first, the situation with regard to the first dorsal ray and the pelvics. The first dorsal ray is gradually shifting towards the head, but the pelvics away from it, and this holds in all three species. If carried on long enough, therefore, the time must come when the first dorsal ray, initially situated at a level well posterior to the pelvics, will come to lie immediately above the pelvics, and then later to be in front of them. This does actually occur in both the herring and pilchard, but in the sprat the process of relative shift appears to cease before the first dorsal ray has quite reached the level of the pelvics. A glance at the diagrams given by Lebour (**2**) will confirm this.

In the case of the first dorsal ray and the anus, we have seen that both tend to move relatively towards the head. Obviously, therefore, it is necessary to take into account the rate at which each is moving. On page 980 it was shown that for unit increase in (L_B–Hd.), the resultant increase in the interval (D^1 to Caud. Ped.) in the pilchard was 1·0 unit. This means that the first dorsal ray is brought 1 mm. farther away from the end of the caudal peduncle. During this time the interval (An. to Caud. Ped.) increases by ·5 mm., which means that the anus is brought farther forward by ·5 mm. The anus and the first dorsal ray have thus become still further separated by (1·0 −·5)=·5 mm. In the case of the sprat, unit increase in (L_B–Hd.) brings about an increase of ·588 mm. in the interval (D^1 to Caud. Ped.), and of ·429 mm. in the interval (An. to Caud. Ped.). The anus and the first dorsal ray in this species thus become further separated by (·588—·429)=·159 mm. as compared with ·5 mm. in the pilchard. Similar working with the herring shows that the additional separation amounts to ·455 mm.

SUMMARY.

Growth in length during the transition from larva to adolescent in the pilchard and sprat has been analysed, and simple straight-line equations deduced from observed data have been used to demonstrate how the different intervals along the dorsal and ventral surfaces of the body alter in length and proportion as development proceeds.

In the herring, pilchard, and sprat at least one of the body-intervals remains for all practical purposes unaltered in length during the transition. In the herring there are two such intervals, namely, (1) the

distance from the back of the brain along the dorsal surface to the insertion of the first dorsal fin-ray, and (2) the distance from the insertion of the pelvic fins along the ventral surface to the anus. In the pilchard and sprat one of the above two remains stationary, but the other increases in length ; in the pilchard the fixed interval is the distance from the back of the brain to the first dorsal fin-ray, and in the sprat it is the distance from the pelvics to the anus.

In the herring and pilchard the greater part of each unit of increase in the length of the body ventrally from the back of the head to the end of the caudal peduncle is added in front of the pelvic fins, whereas in the sprat this is added post-anally.

Along the dorsal surface the first ray of the dorsal fin in the herring, pilchard, and sprat is gradually brought to lie relatively nearer and nearer to the head as development continues. Along the ventral surface the pelvics and anus also undergo alteration in relative position, the pelvics *away* from the head but the anus *towards* it.

In the herring and pilchard the relative forward movement of the first dorsal ray and the relative backward movement of the pelvics is carried on sufficiently to cause a " cross-over " in position on the body, so that whereas initially the first dorsal ray is posterior to the pelvic fins, it is eventually brought to lie anterior to the pelvics. In the sprat this does not occur, and the first dorsal ray remains posterior to the pelvics throughout development.

In the herring, pilchard and sprat a greater proportion of each unit increase in total length is added to the distance from the first dorsal ray to the end of the caudal peduncle than to the distance from the anus to the end of the caudal peduncle, so that as development proceeds the first dorsal ray becomes further and further removed from the anus.

During development the relative lengths of the different body-intervals alter appreciably. In the pilchard the distance from the back of the head to the pelvic fins is at first shorter than that from the pelvics to the anus, but ultimately it becomes the greater of the two. Similarly the distance from the anus to the end of the caudal peduncle commences by being smaller than that from the pelvics to the anus, but eventually comes to exceed it.

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Changes in Length during the Larval Life and Metamorphosis of the Freshwater Eel (*Anguilla vulgaris* Turt.).

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With 5 Figures in the Text.

Introduction.

In other papers (Ford, **2** and **3**) attention was directed to a study of the changes in length which occur during the transformation of the transparent eel-shaped larval clupeoid into the silvery and scaled adolescent fish. The change in position of the anus and fins relative to the vertebræ was considered in connection with the altering proportions of the different body intervals as development proceeds, and simple models were used to illustrate important features.

Now the freshwater eel has its transparent larval stage and metamorphosis. As in the clupeoid, length-proportions change during development, while anus and fins undergo marked migrations with respect to the myomeres. It was therefore thought to be of no little interest to analyse the length-changes during development in a manner similar to that adopted for the herring, sprat and pilchard. Professor Johannes Schmidt most kindly supplied me with material upon which to work, and I have made much use of data already published by him in reports on his great pioneer investigations. I have also drawn extensively from the data given by Dr. Leon Bertin in his paper on the migrations of the anus in the eels during ontogeny. I may be permitted to add that much which follows is suggestive in nature, rather than proven fact. Nevertheless, it should prove of interest in its direct bearing upon questions of fundamental biological importance.

Following the principle adopted by the Italian zoologist Massimo Sella in 1911, Schmidt (**6**, pages 9 and 10) determined the total length and the length of the tail (ano-caudal distance) for each of a representative series of larval *Anguilla vulgaris* Turt. and plotted in a graph the ratio $\left(\frac{\text{total length}}{\text{length of tail}}\right)$ for eight average values of the total length. Schmidt's

figure is reproduced here in Figure 1. It will be seen that for a total length of 15 mm. the above ratio has a value of 4·35, whereas at a total length of 85 mm. it has fallen to 3·20. That is to say, at a total length of 15 mm. the ano-caudal distance is $\frac{15}{4{\cdot}35}$ =3·45 mm., and at 85 mm. it is $\frac{85}{3{\cdot}2}$=26·55 mm.

In the accompanying Figure 2, the line AB measures 15 units of length, of which PB represents 3·45 units. CD is drawn parallel to AB and measures 85 units of length, of which QD represents 26·55 units. The straight line connecting point A with point C is at right-angles to AB

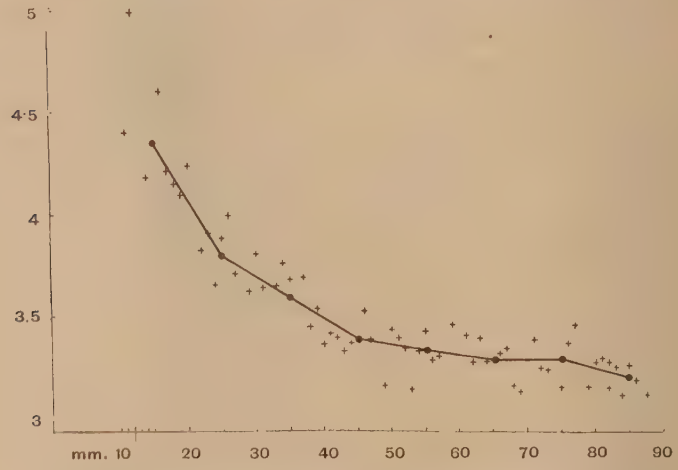


FIG. 1.—European Fresh-Water Eel (*Anguilla vulgaris* Turt.). Showing ratio between total length and length of tail during larval development. (After Schmidt, **6**, page 10, figure 3.)

(or CD) and is of a length equal to the difference between the lengths of AB and CD, namely, 85—15= 70 units. Furthermore, AC is divided into equal intervals of 10 units at points *a* to *f*, and straight lines are drawn parallel to AB (or CD) from each of the points *a* to *f* to intersect PQ and BD.

Now it is obvious that in taking the step of 10 units downwards from point A to point *a*, the length *ag* is greater than AB by an amount equal to one-seventh of the difference (CD- AB) which would be brought about by proceeding the whole distance of 70 units from A to C. Similarly, *pg* exceeds PB by an amount equal to one-seventh of the difference between QD and PB.

Let us assume that this figure is a diagrammatic representation of the manner in which a larval freshwater eel at Schmidt's 15 mm. stage

grows in length to the 85 mm. stage, PB and QD being the corresponding lengths of the tail (ano-caudal distances). The values of the total length and length of tail for successive steps of 10 units along AC in the direction of point C, and of the ratio $\left(\frac{\text{Total length}}{\text{Length of tail}}\right)$ are :—

TOTAL LENGTH.	LENGTH OF TAIL.	$\left(\frac{\text{TOTAL LENGTH}}{\text{LENGTH OF TAIL.}}\right)$
15	3·45	4·35
25	6·75	3·70
35	10·05	3·48
45	13·35	3·37
55	16·65	3·30
65	19·95	3·26
75	23·25	3·23
85	26·55	3·20

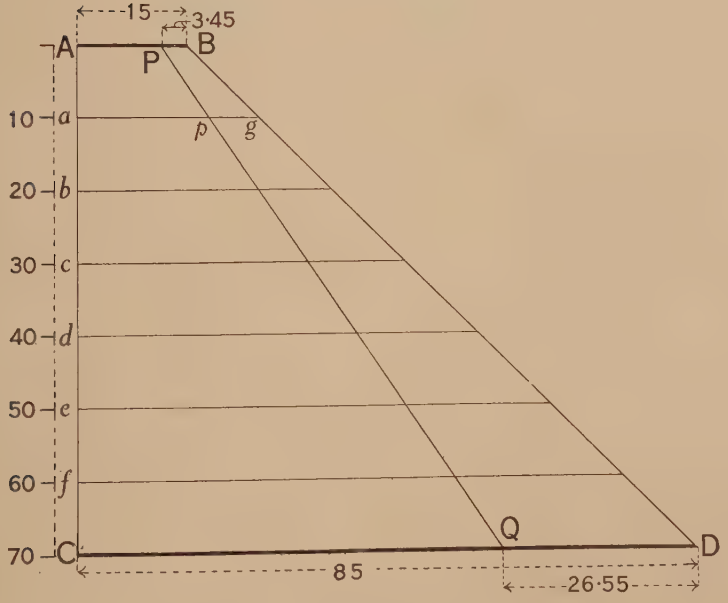


FIG. 2.—For explanation see text page 988.

In Figure 3 the values of the ratio $\left(\frac{\text{Total length}}{\text{Length of tail}}\right)$ have been superimposed on Schmidt's figures (see my Figure 1) and it will be seen that they are quite a fair approximation. In other words, there is evidence for the

assumption that *during growth as a larva, the length of the tail increases uniformly by 3·3 mm. for each increase of 10 mm. in the total length.*

This being so, it can be shown by simple calculation that *the pre-anal length increases uniformly by 6·7 mm. for each increase of 10 mm. in the total length.*

These two deductions may be expressed in the form of equations, thus :—

$$Y_m = 0{\cdot}33\ M - 1{\cdot}50$$
$$Z_m = 0{\cdot}67\ M + 1{\cdot}50$$

where Y_m = Ano-caudal distance at a total length of M millimetres
Z_m = Pre-caudal length " " "

Calculating the value of Y_m when M is 12,

$$Y_{12} = 0{\cdot}33\ (12) - 1{\cdot}5$$
$$= 3{\cdot}96 - 1{\cdot}5$$
$$= 2{\cdot}46$$

The value of the ratio $\left(\frac{\text{Total Length}}{Y_{12}}\right)$ is thus $\frac{12}{2{\cdot}46} = 4{\cdot}88$.

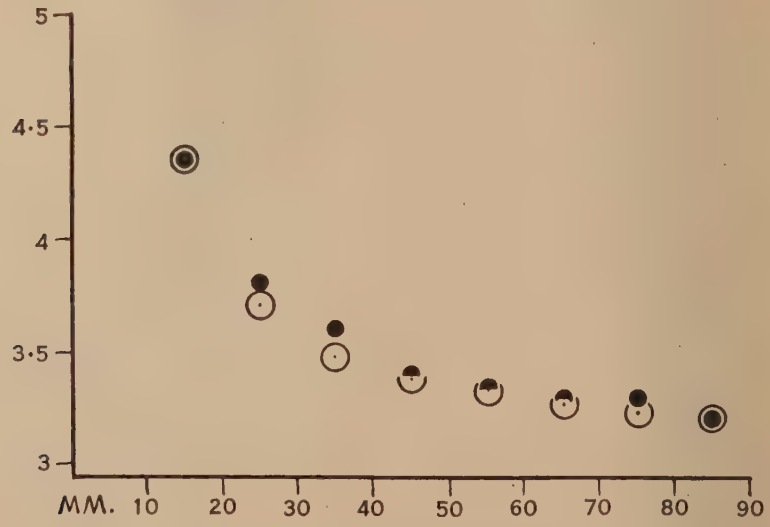


Fig. 3.—Ratio between total length and length of tail during development. Solid black dots are Schmidt's averages (see Fig. 1). Dots within circles are values calculated as explained in text in page 989.

Consulting Schmidt's graph once more (*vide* my Figure 1) it is seen that the solitary record for the ratio at a total length of 12 mm. is about 4·98, a value not greatly different from the above one of 4·88.

The increase in the proportionate length of the tail from the smallest

to the largest larva results in a relative movement of the anus along the ventral surface of the body in the direction of the head. It is important to note, however, that the anus appears not to change its position with respect to the myomere under which it lies. Writing on this point, Bertin (**1**, page 329) summarises the observations by Lea, Grassi and Schmidt on the position of the anus in leptocephaline stages of *Anguilla vulgaris* varying from 8·5 mm. to 83 mm. in length—observations which clearly demonstrate that the anus remains under practically the same myomere throughout. It can be stated, therefore, that growth in length along the vertebral column must agree with that along the ventral surface of the body in being relatively greater post-anally than pre-anally. Otherwise the anus would alter its position with respect to myomeres as growth proceeded. Nor is there any evidence that growth in length along the dorsal surface of the body is of a different nature, since the dorsal fin possesses its full complement of rays at an early leptocephaline stage and its anterior end appears to remain in a constant position relative to the anus throughout leptocephaline life. Thus it may be concluded that *the leptocephalus as a whole increases in length differentially, growth in length being greater post-anally than pre-anally. Neither anus nor dorsal fin alter their position relative to the myomeres.*

Length-Changes during Metamorphosis.

As an introduction to the study of the changes in length which occur during the transformation of the flattened leptocephalus into the rounded elver, I draw attention to a footnote to one of Schmidt's earlier papers (Schmidt, **4**, page 167) on the metamorphosis :—

"It is very interesting to note the gradual change in the nerves which pass to the rays and interspinous rays in the dorsal and anal fins. Though all or at any rate the majority of the rays are present even in the 1st stage, yet both the dorsal and the anal fin are much shorter than in the following stages. In the 1st stage we see how the nerves emerge from the column *far in front of even the beginning of the fins* and are therefore *closely packed together* opposite the fins themselves. In the following stages, when the front part of the fins grows much forwards the nerves become *shortened* and more *separated*, and at the same time their direction relative to the longitudinal axis is quite changed (from being almost parallel or forming a very acute angle at the most with this they become almost perpendicular to it)."

It is a point of great significance that in the fully-grown leptocephalus the rays of the fins are innervated, *not* from the myomeres immediately above or beneath which they lie, but from myomeres some considerable distance in front. Using a binocular microscope, it is possible to trace in

a formalin-preserved leptocephalus the path of an individual nerve from its ending in the fin, forward to the myomere from which it emerges. In a leptocephalus 68 mm. long, having a total of 115 myomeres, I determined that the nerve supplying the first ray of the dorsal fin emerged from the 25th myomere (counted from the head). In contrast with this, the fin ray itself lay immediately above the 65th myomere, that is, forty myomeres farther back towards the tail. Similarly, the first anal ray, lying beneath the 71st myomere, was innervated from the 35th myomere. The observed particulars of this leptocephalus may be summarised thus :—

Length (Snout to end of body) . .	68 mm.
Total number of myomeres . .	115
Snout to 25th myomere . . .	18 mm.
Snout to 35th myomere . . .	25·5 mm.
Snout to 40th myomere . . .	29 mm.
Snout to 46th myomere . . .	33 mm.
Snout to 65th myomere . . .	46 mm.
Snout to 71st myomere . . .	49·5 mm.

In Figure 4A I have given a diagrammatic representation of the leptocephalus, incorporating the above data together with an indication of the paths of the nerves emerging from selected myomeres. I next draw attention to Figure 4B which lies immediately below Figure 4A. Instead of using the outline of a leptocephalus, that of an elver has been employed, the total length being the same. No change has been made in the position of the myomeres, but the nerves emerging from them have been made to pass out at right-angles to the vertebral column, instead of running far back as in Figure 4A. By so doing, however, the position of the dorsal and anal fins with respect to the myomeres, and the proportions of the several body intervals have been greatly altered. The first dorsal ray has come forward forty myomeres to lie over the 25th myomere, while the distance from the snout to the first dorsal ray has become shortened from 46 mm. to 18 mm. The distance from the first dorsal ray to the posterior end of the body, on the other hand, has increased from 22 mm. to 50 mm. Similarly, the anus has advanced thirty-six myomeres and now lies under the 35th myomere, while the distance from the snout to the anus has been reduced from 49·5 mm. to 25·5 mm. The ano-caudal distance has increased from 18·5 mm. to 42·5 mm.

Comparison with the measurements of actual specimens reveals the fact that Figure 4B is a good representation of the proportions of an elver at a length of 68 mm. The significance of this lies in the fact that a close approximation to the elver has been reached by a simple readjustment of the characters of the leptocephalus so that fins and anus are made to lie

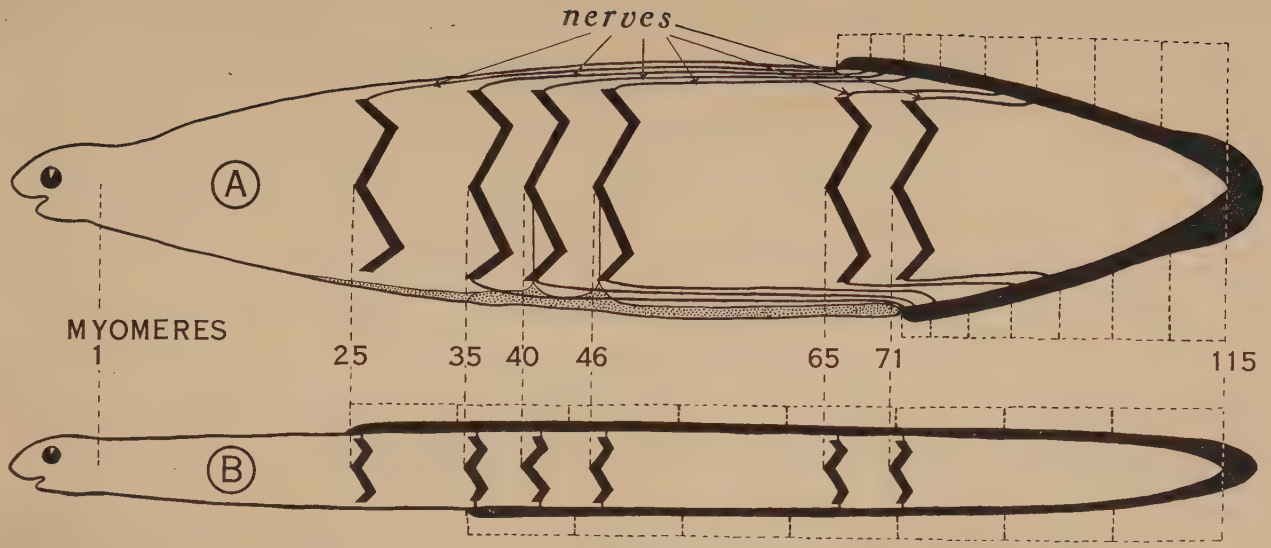


FIG. 4A.– Diagrammatic representation of leptocephalus described in text in page 992. Six myomeres and the dorsal and anal fins are shown in solid black. The nerves from the myomeres to the fins run almost parallel to the spinal column for the great part of their length.

FIG. 4B.—Hypothetical elver deduced from the leptocephalus represented in Fig. 4A. The myomeres remain in the same position as in the leptocephalus, but the nerves are now placed at right-angles to the spinal column. The new positions of the dorsal and anal fins are indicated in solid black.

immediately opposite the myomeres from which they are innervated, instead of being held remote from them.

It is at once realised that the length-changes of the intervals along the dorsal and ventral surfaces of the body during metamorphosis are very different from those which take place while the fish is growing as a leptocephalus. Before metamorphosis, every interval steadily increases in length, growth as a whole being such that the first dorsal ray and anus come gradually to occupy a position relatively nearer the head, although neither changes its orientation with respect to the myomeres. But while the fish is actually undergoing metamorphosis, some intervals increase rapidly in length at the direct expense of others, so that the resultant forward movements of the first dorsal ray and anus are not relative but real. Moreover, in the process of moving, the first dorsal ray and the anus entirely change their position with respect to the myomeres.

Biological Significance of Results.

If Figures 4A and 4B really depict the principle underlying the transformation of the leptocephalus into the elver, save only that no account is taken of the undoubted shrinkage in total length, then there remain many interesting morphological problems to be solved. How do the nerves shorten ? How does the dorsal fin grow forward at the expense of the predorsal portion of the upper surface ? How does the anus come to lie so much further forward ? Some further information concerning the forward movement of the fins and anus can alone be offered here. The examination of the fins of the leptocephalus shows that the fin rays are closely packed anteriorally, but become progressively wider and wider apart towards the posterior end (see Fig. 4A). It may well be, therefore, that the first ray moves forward as the result of the rapid growth of tissues between the fin-rays, causing the fin to " open out " (rather as a collapsible bellows lengthens as it is opened out), until ultimately the rays are uniformly wide apart over a longer length (as suggested in Fig. 4B). Concerning the movement of the anus, Bertin (**1**, page 332) speaks of the autonomous shortening of the digestive tube, and the descriptions given by Schmidt show that the rectal portion of the larval gut gradually shortens during metamorphosis.

It cannot have escaped attention that Figures 4A and 4B raise an interesting question in phylogeny. Which is the more primitive position of the anus—posterior as in the leptocephalus, or anterior as in the elver ? In the leptocephalus, the anus lies a considerable distance behind the myomere from which it is apparently innervated, but moves forward to lie beneath this myomere in the elver. Thus, in effect, Figures 4A and 4B may be interpreted to imply that *the leptocephalus is a specialised form of*

larva in which the anus is temporarily held in a posterior position, moving forward to its true position during metamorphosis.

In this connection, it is instructive to refer to a Table given by Bertin (**1**, page 333, Table VI) in which the French investigator has summarised data concerning the number of pre-anal myomeres and total myomeres in the leptocephaline and adult stages of 15 species of eel. Bertin calculates for each species what he calls "l'amplitude des déplacements anaux," as a convenient measure of the anal displacement during metamorphosis. This amplitude is in the form of a percentage $\left(\frac{a_2 - a_1}{t}\right) 100$, where a_1 and a_2 are the numbers of pre-anal myomeres in the leptocephaline and adult stages respectively, while t is the total number of myomeres for the species in question. It will be easily understood that if the value of this percentage-amplitude is small for a given species, the anus has migrated over a small number of myomeres, compared with the total number of myomeres in the fish. Conversely, a high value of the percentage-amplitude indicates that the anus has moved over a high proportion of the total number of myomeres. It is evident from Bertin's calculations that species differ greatly in the magnitude of the anal displacement during metamorphosis, for the values of his percentage-amplitude range from 0% to 49% in the 15 species for which data are given.

It is, however, important to observe that the value of the percentage-amplitude appears to be dependent upon the position the anus occupies in the leptocephalus. If the anus is far back towards the tail of the leptocephalus in relation to the myomeres, the percentage-amplitude is comparatively high, whereas if the anus is already well forward in the leptocephalus, the percentage-amplitude is small. It is as if in all species there were an attempt during metamorphosis to bring the anus forward to a fixed position in the adult, so that the number of pre-anal myomeres shall be about one-third of the total number. Hence, if in a leptocephalus the anus lies far back, there is a relatively large forward movement of the anus during metamorphosis ; conversely, if in the leptocephalus the anus is well forward, little movement is necessary during metamorphosis to bring the anus to its adult position.

As an example, we may use the data on which Figures 4A and 4B are based. In the leptocephalus of the freshwater eel (Fig. 4A) the anus lies beneath the 71st myomere, but in the elver (Fig. 4B) it is under the 35th. The total number of myomeres is 115. Thus, in the elver the ratio $\left(\frac{\text{number of pre-anal myomeres}}{\text{total number of myomeres}}\right)$ is $\frac{35}{115}$, which is approximately $\frac{1}{3}$. During metamorphosis, the anus has moved from the 71st to the 35th myomere, so that the percentage-amplitude is $\left(\frac{71-35}{115}\right) 100$, or 31%.

Actually, the ratio $\left(\frac{\text{number of pre-anal myomeres}}{\text{total number of myomeres}}\right)$ in the adult is not invariably the same, but differs from species to species. Nevertheless, the values of this ratio tend to group themselves about an average of $\frac{1}{3}$, whereas in the leptocephalus, the corresponding values are widely separated and show no such tendency to group. That this is so is seen in the following Table which summarises Bertin's data for all the species dealt with by him, except *Sphagebranchus cœcus* which he regards as a "mélange probable de deux races" :—

Number of Species having the following values of ratio $\left(\frac{\text{Number of pre-anal myomeres}}{\text{Total number of myomeres}}\right)$:—

	·2–·29	·3–·39	·4–·49	·5–·59	·6–·69	·7–·79	·8–·89	·9–·99
Adult	4	6	4	–	–	–	–	–
Leptocephalus	1	2	4	1	2	2	1	1

Thus, with regard to the position of the anus in relation to the myomeres, the eel species considered by Bertin agree more closely the one with the other in the adult stage than in the leptocephalus—a fact which is of some significance when considering the question of the morphology of the ancestral larva of the eel. It certainly lends support to the view that in the latter the anus lay beneath the myotome from which it was innervated, and was situated in the fore part of the body. Those present-day leptocephalid larvæ which have the anus far back beneath a posterior myotome, although the corresponding spinal nerves arise from an anterior point, must, in this event, be regarded as specialised forms. The degree of specialisation in a given leptocephalus may be estimated by calculating the value of Bertin's "percentage-amplitude" of the anal displacement during metamorphosis. If the value is large, the specialisation is great. To judge from Bertin's data, it would seem that the leptocephaline stages of the species of Synaphobranchus, Congermuræna, Conger, and Anguilla must be considered highly specialised, whereas those of Sphagebranchus, Saurenchelys, Nettastoma, and Muræna are the least so.

Now Schmidt (**5**, page 340) has already suggested the division of the Eel-fishes into two biological groups : (1) those which spawn far from the coasts over great depths, and (2) those which spawn in comparatively shallow water inside or near the 200 m. line. In his first group Schmidt places *Conger vulgaris*, *Conger* (=*Congermuræna*) *mystax*, and *Anguilla vulgaris*. These are species which, as indicated above, have highly specialised larvæ. To his second group Schmidt refers *Ophichthys* (=*Sphagebranchus*) *imberbis*, and *Muræna helena*—and these are species in which the leptocephalus is least specialised.

The conception of a specialised larva is thus brought into direct association with that of an adult habit. Eels which spawn far from the coast

have a specialised leptocephalus, while those which spawn in coastal waters have not. Furthermore, from our discussion of the structure of the ancestral larval eel, we see the possibility that both it and the primitive parent were inhabitants of inshore waters.

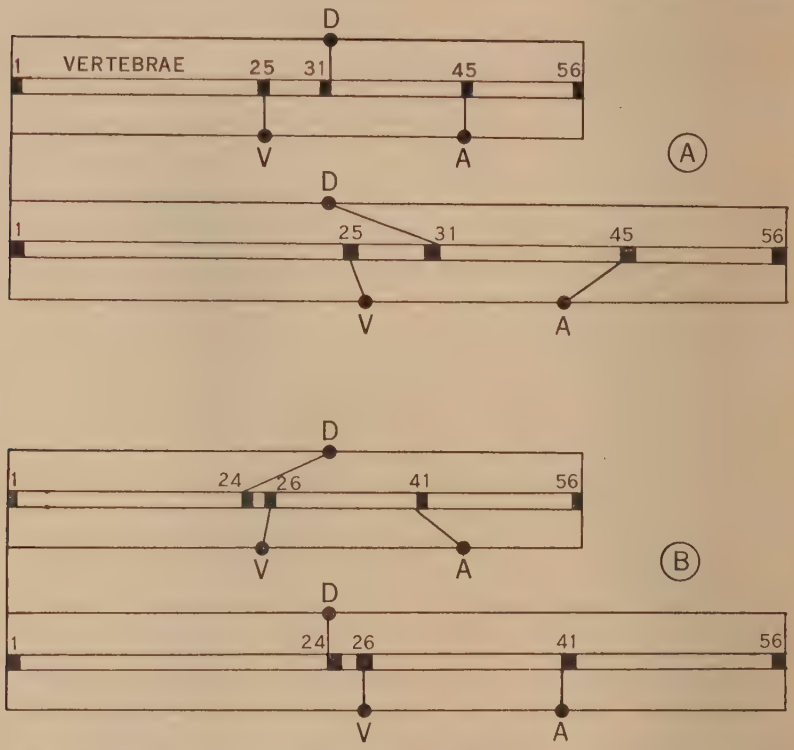
Anguilla and Clupea.

In the growth-changes during early life, the eel, herring, sprat, and pilchard agree in some respects but differ in others. In each instance, the early larva is transparent with the anus situated beneath a posterior myomere. This larva grows and ultimately becomes transformed into an adolescent bearing the familiar characters of the adult. In doing so, the anus is brought forward in relation to the myomeres, and fins undergo a marked change in position. Furthermore, it has been shown in the preceding pages of this paper and elsewhere (Ford, **2** and **3**) that such changes in length-relations can be expressed in terms of the varying rates at which the different body-intervals alter in length as development proceeds.

This generalised agreement between the eel and the clupeoids has its interest, but there are equally important differences. We have seen that the leptocephalus grows in length while still retaining its typical leptocephaline form. Although growth in length is differential, being greatest at the tail-end, the anus and fins do not alter their position relative to the myomeres during this growth as a leptocephalus. The marked readjustment of body proportions whereby the anus and fins assume their adult position takes place as a distinct process of metamorphosis from leptocephalus to elver. In the larval clupeoids, however, differential growth in length is such that the anus and fins steadily change their position relative to the myotomes as the larva increases in length. This amounts to a process of gradual transformation into an adolescent of larger size, as opposed to one in which a larva first grows as a larva, and then changes to an adolescent by a distinct act of metamorphosis. The end result may be the same in the two cases, in that the anus is brought forward to its adult position, but the "timing" of the developmental events leading to the end result is different. In the clupeoid, growth in total length and anal migration proceed simultaneously; in the eel, growth in total length takes place while the fish is yet a leptocephalus, and the anal migration is postponed until later.

We know that the larval life of the eel is very lengthy compared with that of the clupeoid, and we have discussed the probability that the leptocephalus is a specialised larval form, organised in a manner suited to the conditions imposed by the oceanic spawning of the eel. We have suggested that the position of the anus in the after part of the body of the

leptocephalus is one of the special modifications, and have referred to the manner in which the spinal nerves have become drawn-out so that they end at a point much farther back than their origin in the spinal cord. What is the situation in the young of the clupeoid ? What can we learn



Figs. 5A and 5B.—*Clupea harengus*. Possible alternatives in innervation of young stages. (See text in page 999.)

D = First ray of dorsal fin. V = Pelvic fins. A = Anus.

5A. Upper. Length 88 units.
Nerves assumed to leave vertebral column at right-angles.

5A. Lower. Length 119·5 units.
Nerves originate and end at points identical with those shown in Fig. 5A. Upper.

5B. Lower. Length 119·5 units.
Nerves assumed to leave vertebral column at right angles.

5B. Upper. Length 88 units.
Nerves originate and end at points identical with those shown in Fig. 5B. Lower.

from a study of the nerves in successive stages of development ? Unfortunately, it is not possible by simple gross examination as with the leptocephalus to trace the spinal nerves of the clupeoid larva from source to end point, nor have I, as yet, found opportunity to conduct the necessary

micro-examination which would provide an answer to this question. But there are two alternatives which are well worth considering here. The spinal nerves of the clupeoid larva either pass out at right angles to the spinal cord so that they innervate parts of the body immediately opposite to their origin, or, failing this, they run out obliquely to innervate parts of the body which are not immediately above or below their origin.

Consider these alternatives as applied to the herring. Given the following data concerning a post-larva and an adolescent (from Ford, 2) : —

BODY INTERVAL.	POST-LARVA.	ADOLESCENT.
	(Units of Length.)	
Body Length	88·0	119·5
Head to 1st ray of Dorsal Fin . .	49·0	49·0
Head to Pelvics	39·0	54·9
Pelvics to Anus	30·5	30·5

In Figure 5A, upper, constructed in accordance with the above data for the post-larva, the nerves supplying certain points have been represented by lines drawn at right angles from the vertebræ. Figure 5A, lower, is constructed on the data for the adolescent, and lines have been drawn linking the same origin and end-point of nerves shown in Figure 5A, upper. It is seen that in the adolescent the path of each nerve is now oblique to the vertebral column, whereas in the post-larva it was at right-angles to it. In this case the adolescent is, so to speak, a distortion of the post-larva.

The other alternative is illustrated in Figures 5B, upper, and 5B, lower. It is here simpler first to construct a representation of the adolescent, as in Figure 5B, lower, inserting nerves at right-angles to the vertebral column. The figure for the post-larva (Fig. 5B, upper) in next drawn with the nerve origins and endings as in Figure 5B, lower. It is seen that it is now the post-larva and not the adolescent in which the nerves take an oblique course. In other words, the post-larva is now a distortion of the adolescent.

Which of these two is to be regarded as approaching the true state of affairs is a matter which can be settled by dissection, and is worth investigation, since it is of direct interest in the study of the evolutionary history of the fishes concerned. It is hoped that further investigation on this question will be made in the near future.

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Relative Growth of the abdomen and the carapace of the Shore-Crab *Carcinus maenas.**

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With 5 Figures in the Text.

Introduction.

In 1920 the senior author undertook some experiments on shore-crabs (*Carcinus maenas*) to see whether the development of their secondary sexual characters was altered by thyroid or pituitary feeding. To carry these out, it was first necessary to find the normal relative growth-rate of these characters, and a number of measurements were undertaken for the purpose. Some further measurements were made by the senior class in Zoology at Oxford as an exercise. By then, however, it had become apparent that the interpretation of the measurements of the normal crabs offered more interesting problems than the endocrine experiments, which turned out to be wholly negative, but that more accurate methods of measurement were necessary for their solution. Accordingly the junior author undertook to devise and carry out these measurements.

The common shore-crab can be strongly recommended as material for many types of investigation. For biometric work it offers the advantage of a hard skeleton. As class-material in biometric study it is cheap and easily handled. It is good for the study of growth-problems, since it has an " unlimited " growth and since the cast moults can be measured with as great an accuracy as the whole animal. It is easily kept in captivity far from the sea. Specimens up to 1 or 2 inches' carapace-length, kept in $\frac{1}{2}$ to 1 inch of sea-water in covered finger-bowls or (cheaper !) in tongue or potted-meat jars, need only to be fed and have their water changed twice a week ; and larger specimens can equally well be kept if larger receptacles are provided. A few preliminary trials indicate that injections into the muscles or haemocoel can be satisfactorily made through the

* Studies in Heterogonic Growth, No. V.

arthrodial membranes at the base of the limbs, or between thorax and abdomen. The crabs can be kept healthy on meat or still better on fish, but will accept, and thrive on, a large variety of foods. Finally, it is an abundant and easily-obtained species, and it is apparently rather variable.

All specimens with a Sacculina externa were of course rejected, since sacculinisation is known to increase the breadth of the abdomen in both sexes. Some specimens with Sacculina interna are no doubt included in the population measured; however, the error thus introduced will not be large, partly because such individuals are not common, partly because sacculinisation will not have had time to produce much effect on the abdomen before the parasite becomes external.

In larger individuals, the measurements were made with fine callipers. This gives a slight positive error, which is, of course, relatively larger in smaller specimens. Accordingly in these (up to about 9 mm. carapace length) the size of the parts to be measured was indicated on white paper by means of a camera lucida and a dissecting microscope, measured with a ruler, and the absolute size calculated from the known magnification.

Carapace length was measured in the mid-dorsal line. Abdomen-breadth refers to the breadth at the joint between the 5th and 6th abdominal segments.

Results.

The number of crabs accurately measured was 678. Most of them came from Plymouth, at various seasons of the year. In order to obtain some specially large animals, 53 large crabs were procured from Essex: as these came from another locality, their measurements are recorded separately. The distribution of the crabs was as follows:—

Plymouth crabs: unsexable (externally), 74; males, 270; females, 281. Essex crabs: males, 22; females, 31. The results of the measurements are shown in the subjoined Tables I and II. The individual measurements are not given, but the means for classes according to carapace-length.

Graphic presentation of the results is given in Figures 1 and 2, where the relative abdomen breadth (percentage of carapace length) is plotted for each sex.

It was not considered worth while calculating the standard deviation for each of these means. To give some idea of the range of variability, however, the extreme variants in either direction have been given (Table II).

It will be noted that in almost every class, the range of variation shown by the females is considerably greater than that of the males; in the one exception, they are nearly equal. This is to be expected, since slight differences in the onset of the rate of differential female abdomen-growth would make a considerable difference in the ratios.

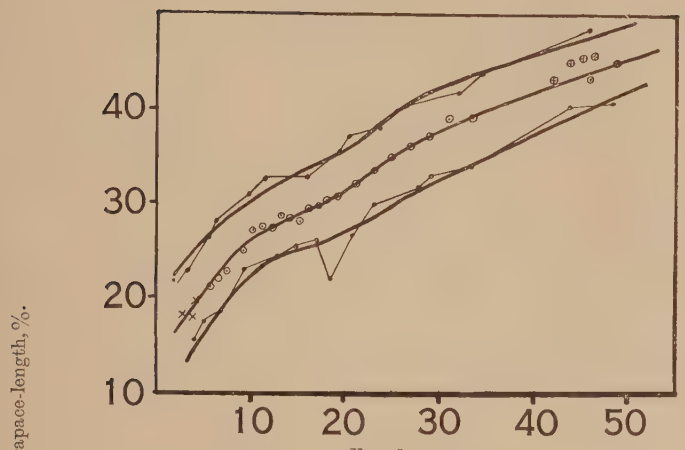

FIG. 1.

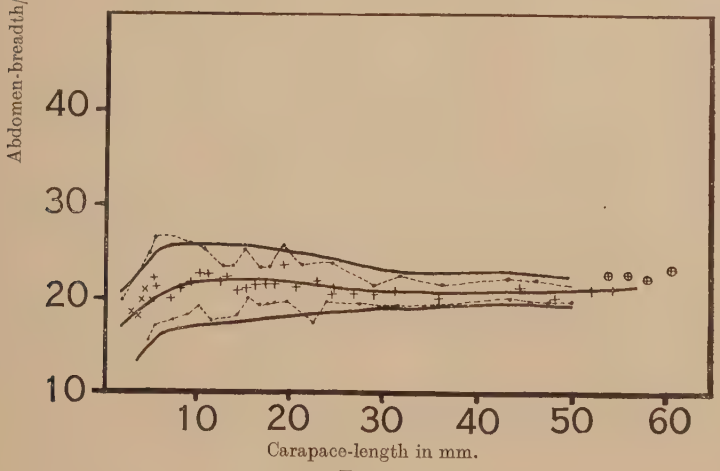

FIG. 2.

FIGS. 1 and 2.—The relative abdomen-breadth of female and male *Carcinus maenas*, against carapace-length.

× unsexable (in both figures).
⊙ Plymouth females. ⊗ Essex females.
\+ „ males. ⊕ „ males.

The extreme variants are also given, together with smoothed curves for the probable range of variation.

TABLE I.

ABDOMEN-BREADTH RELATIVE TO CARAPACE-LENGTH IN CARCINUS MAENAS.

(*a*) UNSEXABLE (74 specimens).

Class :— Carapace-l. (mm.)	Σ	Mean car.-l. (mm.)	Mean abd.-br. (mm.)	Mean $\frac{\text{abd.-br.}}{\text{car.-l.}}$ %
2·4–3·4	13	3·09	0·578	18·7
3·5–3·9	22	3·80	0·680	17·9
4·0–4·5	20	4·22	0·823	19·5
4·5–4·9	11	4·76	0·979	20·6
5·0–5·5	8	5·19	1·019	19·6

(*b*) MALES (292 specimens).

Plymouth crabs (270 specimens).

4·75– 6·0	11	5·50	1·238	22·5
6·0 – 7·0	6	6·37	1·224	19·2
7·0 – 8·0	16	7·51	1·48	19·7
8·0 – 9·0	14	8·41	1·75	20·8
9·0 –10·0	15	9·37	2·01	21·4
10·0 –11·0	10	10·36	2·31	22·3
11·0 –12·0	26	11·31	2·56	24·4
12·0 –13·0	12	12·63	2·72	21·5
13·0 –14·0	18	13·32	2·93	22·0
14·0 –15·0	15	14·34	2·95	20·6
15·0 –16·0	10	15·43	3·21	20·8
16·0 –17·0	14	16·35	3·47	21·2
17·0 –18·0	16	17·51	3·71	21·2
18·0 –19·0	6	18·34	3·93	21·4
19·0 –20·0	11	19·37	4·43	23·4
20·0 –22·0	13	20·73	4·56	21·0
22·0 –24·0	19	22·93	4·97	21·7
24·0 –26·0	9	24·58	4·95	20·2
26·0 –28·0	4	26·69	5·44	20·4
28·0 –30·0	3	29·03	5·93	20·4
30·0 –31·0	4	31·16	6·48	20·8
35·0 –37·0	4	35·75	7·13	19·9
43·0 –47·0	5	44·54	9·42	21·1
47·0 –51·0	4	48·35	9·63	19·9
52·0 –57·0	5	54·44	11·46	21·0

TABLE I (*continued*).

Essex crabs (22 specimens).

Class :— Carapace-l. (mm.)	Σ	Mean car.-l. (mm.)	Mean abd.-br. (mm.)	Mean $\frac{\text{abd.-br.}}{\text{car.-l.}}$ %
53·0 –55·0	9	53·98	12·09	22·4
55·0 –57·0	6	56·00	12·58	22·4
57·0 –59·0	4	57·87	12·73	22·0
60·0 –62·0	3	60·57	14·00	23·1

In plotting, the first 2 classes of the Plymouth males have been combined (Fig. 2).

(*c*) FEMALES (312 specimens).

(by 1 mm. carapace-length classes, except that the 8 mm. and 9 mm. classes have been combined, as there was only 1 specimen in the 8 mm. class.)

Plymouth crabs (281 specimens).

Σ	Mean Carapace-length mm.	Mean Abdomen-breadth mm.	Mean $\frac{\text{Abdomen-br.}}{\text{Carapace-l.}}$ %
12	5·56	1·16	20·9
16	6·52	1·45	22·2
14	7·41	1·67	22·6
12	9·32	2·30	24·7
15	10·37	2·80	27·0
16	11·35	3·11	27·4
17	12·33	3·37	27·3
23	13·29	3·81	28·6
19	14·35	4·06	28·3
12	15·31	4·29	28·0
19	16·35	4·82	29·4
15	17·36	5·15	29·6
15	18·16	5·48	30·2
7	19·34	5·93	30·6
6	20·33	6·72	32·1
10	21·51	6·82	
12	22·40	7·54	33·6
8	23·34	7·76	
6	24·33	8·33	35·0
5	25·52	9·16	
10	26·45	9·59	35·9
6	27·65	9·79	

TABLE I (*continued*).

Σ	Mean Carapace-length mm.	Mean Abdomen-breadth mm.	Mean Abdomen-br. / Carapace-l. %
2	28·45	10·40	37·2
3	29·30	11·03	
4	30·55	11·62	38·8
4	31·49	12·46	
3	32·37	12·75	38·9
5	33·32	12·70	
4	34·33	13·56	
1	43·50	17·50	43·2
2	45·30	19·20	
1	46·30	19·90	
1	50·20	23·70	
	Essex crabs (31 specimens).		
4	42·10	18·15	43·1
7	43·56	19·54	44·8
6	44·38	19·88	44·8
6	45·28	20·50	45·3
4	46·43	21·13	45·5
4	48·75	21·88	44·7

In plotting (Fig. 1), the Plymouth females have been combined into 2 mm. classes from carapace-length 20 to 33 mm., and the last 4 classes combined into one; and the 8 and 9 mm. classes have been combined in the table, since only one crab of the 8 mm. class was found. Of the Essex females, the 43 and 44 mm. classes, and also the 45 and 46 mm. classes, have been combined in plotting.

When the means are plotted on a double logarithmic grid (Fig. 3) further information is acquired. In general, the points lie along good approximations to straight lines, indicating that constant differential growth-ratios are maintained over long periods of the life-history. The curve for the unsexed is prolonged by that for the females up to a carapace-length of between 17 and 20 mm.; during this period the growth-coefficient of the abdomen is about 1·26. From this point onwards, the relative growth of the female abdomen increases, and remains steady at about 1·42 up to the largest specimens found.

From the moment the sexes are distinguishable, the male curve diverges from the female. It also shows two phases; the growth-coefficient for the first is about 1·07, for the second about 0·94. It is more difficult to

distinguish the exact size at which the change in growth-ratio occurs in the males than in the females, since the male points are more irregular. At any rate it takes place at approximately the same phase in the life history, certainly between 14 and 21 mm. carapace-length. If, as would seem probable on *a priori* grounds, the change occurs at sexual maturity, we should expect there to be a considerable variation in the absolute size at onset.

TABLE II.

Maxima and Minima for Abdomen : Carapace Ratio.

FEMALES.

Maxima.				Minima.			
Class. Car.-l., mm.	Carapace-length	Abd.-br. / Car.-l. %		Class. Car.-l., mm.	Carapace-length.	Abd.-br. / Car.-l. %	
> 40	45·8	48·2		> 40	48·3	40·6	
	44·4	48·1			43·5	40·2	
30–35	31·75	41·75		30–35	33·5	34·0	
	34·5	43·5		25–30	27·5	31·8	
25–30	25·6	38·3		20–25	21·5	28·4	
	26·25	40·4			22·0	26·4	
20–25	20·25	37·0		17–20	17·0	23·6	
	23·5	37·9			18·25	21·9	
17–20	19·5	35·5		15–17	14·75	25·4	
15–17	16·0	32·8		11–13	11·0	24·4	
13–15	14·75	30·5		9–11	9·1	22·8	
11–13	13·0	30·8		5–9	5·8	20·7	
11–13	11·50	32·6					

UNSEXED.

4·6–5·5	5·5	27·3	4·6–5·5	5·0	17·7
4·0–4·5	4·1	26·4	4·0–4·5	4·1	15·9
3·5–3·9	3·6	22·2	3·5–3·9	3·9	15·4
3·0–3·4	3·4	21·6	3·0–3·4	3·1	16·1
3·0	2·4	19·4			

Up to the size of 22 mm. carapace-length, the variation-curves for male and female overlap, the least male-type males having broader abdomens than the least female-type females.

It is of considerable interest to find the growth-ratio during the earliest (unsexed) phase apparently identical with that of the first female phase.

It would seem difficult to escape the conclusion that the growth-ratios of the male abdomen change not once but twice, the first time at carapace-length about 5 mm., whereas that of the female only changes once. It

would however be desirable to check this result by measurement of specimens unsexable externally, followed by sexing by means of dissection.

After a large number of measurements had been taken, it was realised that perhaps carapace-breadth would have been a better means of absolute

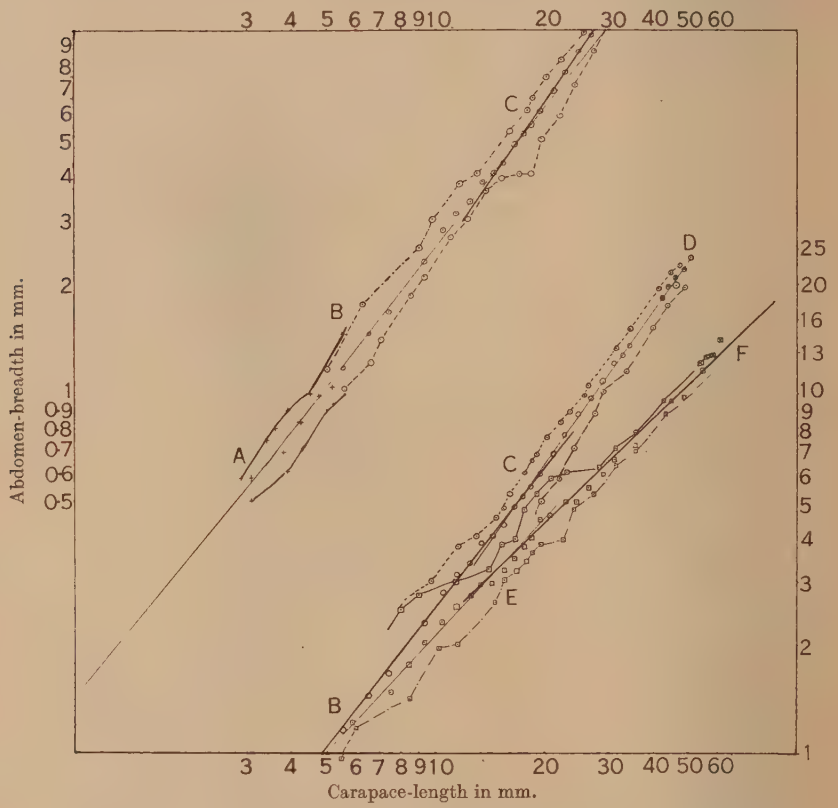

FIG. 3.—Double logarithmic plot of abdomen-breadth against carapace-length in *Carcinus maenas*. Above (abdomen-breadthscale to left) the curve for unsexable crabs (+) and the first part of that for females (⊙). Below (abdomen-breadth scale to right) the curves for females (⊙) and males (⊡).

A–B, unsexable period.
B–C, 1st female period.
C–D, 2nd female period.
B–E, 1st male period.
E–F, 2nd male period.

In addition to the means, the extreme variants are also given.

size than carapace-length, since in that case one would be comparing two breadths, instead of a breadth with a length. Accordingly, in the case of the last 201 animals measured, the greatest carapace-breadth was taken in addition to the other two measurements. The following results were found.

TABLE III.

THE RELATION OF CARAPACE-LENGTH TO CARAPACE-BREADTH IN 201 SPECIMENS FROM 2 TO 52 MM. CARAPACE-LENGTH.

Class; car.-l., mm.	Σ	car.-l.	car.-br.	car.-br./car.-l. % mean	range.	Σ	car.-l.	car.-br.	car.-br./car.-l. % mean	range.
	Unsexed (50 specimens).									
2–4	18	3·54	4·13	116·7	(103–136)					
4–6	32	4·69	5·56	118·5	(110–125)					
	Males ♂ (66 specimens).					Females ♀ (85 specimens).				
5–10	24	7·35	8·88	121·0	(112–127)	37	6·88	8·37	121·6	(116–132)
10–15	12	13·80	17·19	124·6	(121–130)	10	12·80	15·97	124·8	(120–130)
15–20	12	17·13	21·96	128·2	(126–130)	21	17·90	23·37	130·0	(125–135)
20–25	8	22·40	29·33	130·5	(128–133)	9	22·19	28·98	130·6	(129–132)
25–30	5	26·60	34·34	129·8	(128–133)	3	26·77	35·07	131·0	(130–133)
30–35	1	31·6	41·1	130·1		3	32·0	31·3	129·1	(127–133)
35–40	2	36·4	48·2	132·5		—	—	—	—	
45–55	2	49·7	66·8	134·5		2	51·7	67·8	131·1	

These results have been plotted in two ways: (*a*). (Fig. 4) the relative carapace-length against absolute carapace-length; (*b*) absolute carapace-breadth against absolute length, on a double logarithmic grid. The mean percentage (relative) carapace-breadth increases from 116·7 to nearly 135, an increase of about 15%. The means for the females are at first slightly above those for the males, but much greater numbers would be needed to establish this point significantly. (The numbers for the last points, from class 25–30 mm. onwards, are too certainly small to warrant attaching any importance to sexual differences.)

The range of variation in percentage carapace-breadth is considerable,

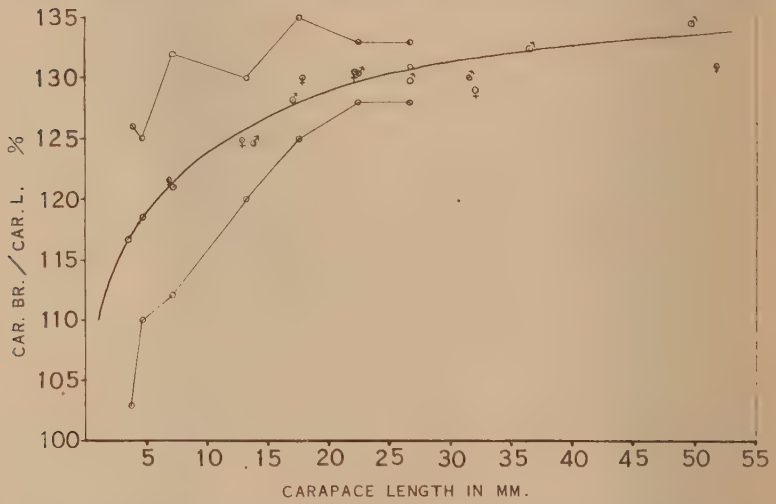

Fig. 4.—The alteration in relative carapace-breadth with absolute size. The mean values are given for unsexed, ⊙; females, ♀; and males, ♂. In addition the extreme variants, irrespective of sex, are given up to 28 mm. carapace-length.

being 23 for the smallest specimens, and diminishing to 10, in spite of increased numbers, by the 15–20 mm. class.

On the double logarithmic plot (not reproduced) the points fall on to a very close approximation to a straight line, whose inclination gives a value of 1·06 or just below, for the growth-coefficient of carapace-breadth relative to carapace-length. The points for the last three classes fall slightly below the line, giving a growth-coefficient of unity or slightly over; but they comprise too few specimens to enable us to say for certain that there is any real change in the growth-ratio. Extrapolation of the curve downwards enables us to say that at carapace-length 1 mm., the carapace-breadth should be 1·1. This is indicated on the graph for percentage values.

By using the ratios thus obtained as a correction coefficient, it was possible to prepare a graph (not here reproduced) of the relation between abdomen-breadth and carapace-breadth. As was to be expected from the regularity of the relation between carapace-breadth and carapace-length, this graph was essentially similar to those constructed with carapace-length as the standard.

Discussion.

Extended discussion of these results need not be undertaken here, as the theoretical bearings have been discussed elsewhere (Huxley 1927, etc.).

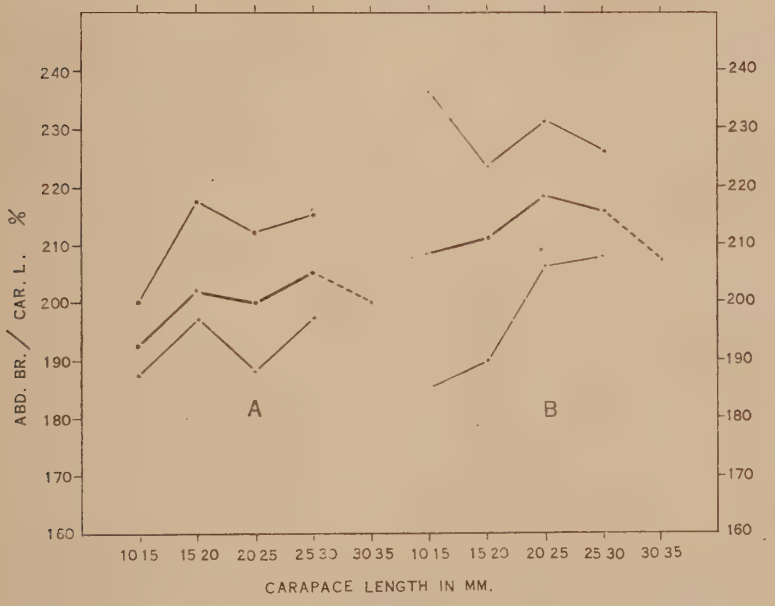

Fig. 5.—Seasonal variation in abdominal-breadth. Relative abdomen-breadth of male crabs from Plymouth (mean and range), A, in June, on left; B, in October, on right.

We need only stress the fact that in regard both to abdomen-breadth and carapace proportions, Carcinus has no fixed proportions in either sex, although the changes are more obvious in regard to the female abdomen. Since these animals show unlimited growth, there is not even any set of proportions which we can regard as adult or in any sense definitive. All that is fixed about the proportions of the shore-crab is their rate of change, as given by the growth-coefficients of the various organs.

One point deserves mention. In the Fiddler crab, Uca, the female abdomen grows heterogonically through all the early periods of life,

but after a certain time approaches the isogonic type (Morgan, 1923, and Huxley, 1924). The difference between this species and Carcinus is presumably correlated with the fact that in Uca the abdomen of the large females touches the bases of the legs on either side, whereas in Carcinus in the largest specimens I have seen there is still a considerable gap between lateral margin of abdomen and leg-bases—but by what means the abdomen growth is slowed down at the correct moment we do not know.

The matter is complicated, however, by the fact that in Pinnotheres, the pea-crab, the female abdomen continues to increase in relative size throughout life, even though it comes to overlap the bases of the legs very considerably (see the illustrations in Atkins, 1926 ; and Huxley, 1931).

A minor point is the apparent seasonal variation in the relative size of the male abdomen. The following table gives the results for 73 of the male crabs measured late in the investigation, all of which were taken at Plymouth, but 23 in early June, 50 in October.

The results are set forth in Table IV and Figure 5.

It will be seen that the October males have a consistently higher relative abdomen-breadth. As possible explanation of this, it may be suggested that after the breeding season a non-breeding phase sets in in which the male characteristics tend somewhat in the direction of the female ; this is known to occur in regard to certain species of Inachus as regards the male chela (G. W. Smith, 1906, and Shaw, 1928).

TABLE IV.

Relative abdomen br. $\left(\frac{\text{abd. br.}}{\text{car-l.}}\right)$ %, males.

Classes (mm. car. l.)	June. No.	Mean.	Max.	Min.	October. No.	Mean.	Max.	Min.
10–15	6	19·4	20·0	18·8	21	20·8	23·6	18·6
15–20	6	20·2	21·7	19·7	13	21·0	22·3	19·0
20–25	5	20·0	21·2	18·9	11	21·8	23·2	20·6
25–30	5	20·6	21·5	19·7	4	21·6	22·6	20·8
30–35	1	20·1			1	20·7		

Feeding Experiments.

With regard to the feeding experiments, mentioned in the introduction, a few comments may be made in spite of the results being wholly negative.

Table V gives the percentage increases at the moults for the three classes of the thyroid-fed, the pituitary-fed, and the control animals.

At first sight it may seem surprising that crabs fed exclusively for periods of 6 to 12 months and over on fresh ox-thyroid or ox-pituitary should exhibit no changes in growth or other characteristics as compared with controls fed on fresh fish. As regards the thyroid, however, the work of

Romeis (1925) has since shown that we should not expect any results of an endocrine nature to be obtained from feeding experiments with crustacea. He found that in the crayfish the active principle of the thyroid hormone was destroyed by the digestive enzymes, instead of being absorbed unchanged as in vertebrates, and we may presume that the same holds true for other Decapoda. Any experiments on the effect of thyroid upon crustacea should therefore be made by means of injection of extracts.

TABLE V.

GROWTH MADE BY CRABS FED ON FRESH FISH, FRESH OX-THYROID AND FRESH OX-PITUITARY, OVER PERIODS OF 1 OR 2 MOULTS.

		Initial carapace-length. mm.	1st moult, % increase.	2nd moult, % increase.
CONTROL		9·0	23·4	27·0
		10·9	20·2	19·8
		11·6	24·4	25·2
		14·1	26·2	23·6
		15·2	22·9	21·3
		14·0	18·6	—
	Mean		22·6	23·4
THYROID		9·1	27·6	24·1
		14·4	21·5	20·6
		15·5	25·8	23·6
		16·2	27·6	10·6
		17·2	26·8	17·0
		14·1	20·6	
		15·7	20·4	
		16·1	26·1	
		18·5	18·8	
		19·2	18·7	
	Mean		23·4	19·2
PITUITARY		13·7	23·5	25·4
		15·0	23·4	20·6
		15·4	25·4	21·2
		18·4	23·4	20·7
		13·8	24·4	
		14·6	24·4	
		15·0	25·3	
		16·9	23·6	
		17·9	24·6	
	Mean		24·2	22·0

The mean increase in carapace-length for all first moults is 23·5%; for all second moults, 21·5%; for all moults taken together, 22·8%.

Przibram has contended that arthropods in general tend to double in weight (bulk) at each moult. For an animal remaining constant in all its proportions, the percentage increase in a linear dimension needed to fulfil this condition is almost exactly 26%: ($\sqrt[3]{2} = 1{\cdot}26$). In Carcinus, we have already seen that carapace-breadth is increasing a little faster than carapace-length; it is also certain from analogy with other crustacea that the growth of at least the larger limbs will be at a slightly higher rate than that of the carapace. The increase of carapace-length at each moult needed to give a doubling in weight should therefore be slightly less than 26%. The figures actually found thus support Przibram's contention.

SUMMARY.

1. In regard to relative abdomen-breadth and relative carapace-breadth, the common shore-crab *Carcinus maenas* in both sexes exhibits a continuous change of proportions throughout life, from small animals of 2 mm. carapace-length to the largest obtainable (over 50 mm. carapace-length).

2. The parts measured exhibit constant differential growth-ratios (growth-coefficients) relative to carapace-length taken as standard, over long periods of the life-history.

3. The approximate growth-coefficients are as follows, relative to carapace-length:

Carapace-breadth, 1·06. This appears to be constant throughout the range of size measured, with a possible decline to nearly unity at large sizes.

Abdomen-breadth.

Crabs unsexable externally (2–6 mm. carapace-length), 1·26.
Females: early phase, 1·26; late phase, 1·42.
Males: early phase, 1·07; late phase, 0·94.

The early phase begins as soon as the sexes can be distinguished. The onset of the late phase may be presumed to be correlated with sexual maturity. In females it occurs certainly between 17 and 20 mm. carapace-length, in males between 14 and 29 mm.

4. There are indications of seasonal and local differences in relative abdomen size.

5. Feeding crabs of 9 to 22 mm. carapace-length solely on fresh ox-thyroid or fresh ox-pituitary for periods up to a year produces no change in growth-rate or in secondary sexual characters as against controls fed on fresh fish.

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Apparatus for the Photo-Electric Measurement of Submarine Illumination assembled for the U.S.A. Research Ship *Atlantis.*

By

D. C. Gall and W. R. G. Atkins.

With 4 Figures in the Text.

Introduction.

Shelford and Gail (1922) first used photo-electric cells for submarine photometry in the Puget Sound. Poole (1925) produced an apparatus which was suitable for use on small ships in the open sea. This apparatus was used near Plymouth by Poole and Atkins (1926, 1928, 1929) with various minor alterations in design which rendered it more reliable and less subject to errors due to electrical leakage. The object of these researches was to study illumination in relation to photosynthesis, but Russell perceived their bearing on his own work on the phototropic movements of the zooplankton. When considering the research programme of the *Atlantis*, Dr. George L. Clarke decided that it would be of value to carry out photo-electric measurements of submarine illuminations in connection with his work on vertical distribution of the plankton. A request was accordingly made by Dr. H. B. Bigelow, Director of the Wood's Hole Oceanographic Institute, that such an apparatus should be assembled under our direction. The apparatus here described has been constructed closely upon the lines of H. H. Poole's original apparatus as modified in 1928. Special attention, however, has been given to the elimination of electrical leakage. Furthermore, J. H. J. Poole (1928) devised an apparatus in which rate of flash of a neon lamp was used to measure light. This rate of flash method is one of integration and not an instantaneous measurement as is that of H. H. Poole. The value of the neon lamp method lies in the fact that light under the water is extremely variable near the surface owing to the motion of the waves. This method of measuring under-water illumination was used in Lough Bray, Co. Dublin, by J. H. J. and H. H. Poole (1930), and has since been used at sea by Poole and Atkins (1931). It seemed advisable to include a neon lamp photometer in the outfit so that errors in determining the loss of light on entering the water might be minimised.

The Potentiometer Apparatus.

This equipment is a functional copy of that in use at Plymouth. The original apparatus had been assembled from various parts, and in reproducing this the opportunity was taken of making the constructional improvement possible in apparatus being designed for a specific purpose. One of the chief difficulties is that of insulation, because photo-electric measurements are essentially measurements of very high resistance. During sea fog, surface leakage becomes a serious matter, and in designing the present apparatus this was one of the principal problems to be overcome. The measuring apparatus shown in Figure 1 consists of a potentiometer with associated circuits to measure the current passing through the photo-electric cells under different degrees of illumination. The potentiometer has two dials, the main dial consisting of 18 steps of 0·1 volts, and the slide-wire subdivided into calibrated divisions of 0·001 volts, readable by estimation to 0·0001 volts. A reducing ratio extends the range to one-tenth of the above values, so that the total range of measurement covered is from 1·9 volts to 10 micro-volts. The current passing through the photo-electric cell is measured by observing the volt drop upon a standard resistance in series with it. This resistance consists of 10 steps of 10,000 ohms each, giving a total of 100,000 ohms. With this combination photo-electric currents from $1{\cdot}9\times10^{-4}$ down to 10^{-9} ampere can be measured. To prevent surface leakage at the potentiometer this instrument is totally enclosed in an air-tight metal box. The spindles operating the dials are brought out through glands of a type which has been found successful in somewhat similar instruments for field use. The terminals are all of a special " petticoat " type, hermetically sealed into the metal case with a special high insulating compound. The advantage of this petticoat type of terminal is that the surface leakage path, besides being very long, is shielded from light, and constructed so that condensation from the atmosphere cannot easily form upon the inside surface of the petticoat. The metal top of this instrument is sealed to the case with a rubber gasket between the two metal surfaces. A drying chamber containing calcium chloride is provided to remove the residual moisture inside the instrument. This same type of sealed construction is used for the 100,000 ohms subdivided standard resistance, by means of which the photo-electric currents are measured. A further advantage resulting from this form of metallic construction is that no direct leakage can take place between terminals should the insulation at any time be lowered, but must first leak to the metal case. This allows of employing a " guard " circuit to deflect such leakage away from the measuring circuit. To provide for this, each metal case is highly insulated from the metal lining to the sea-chest containing

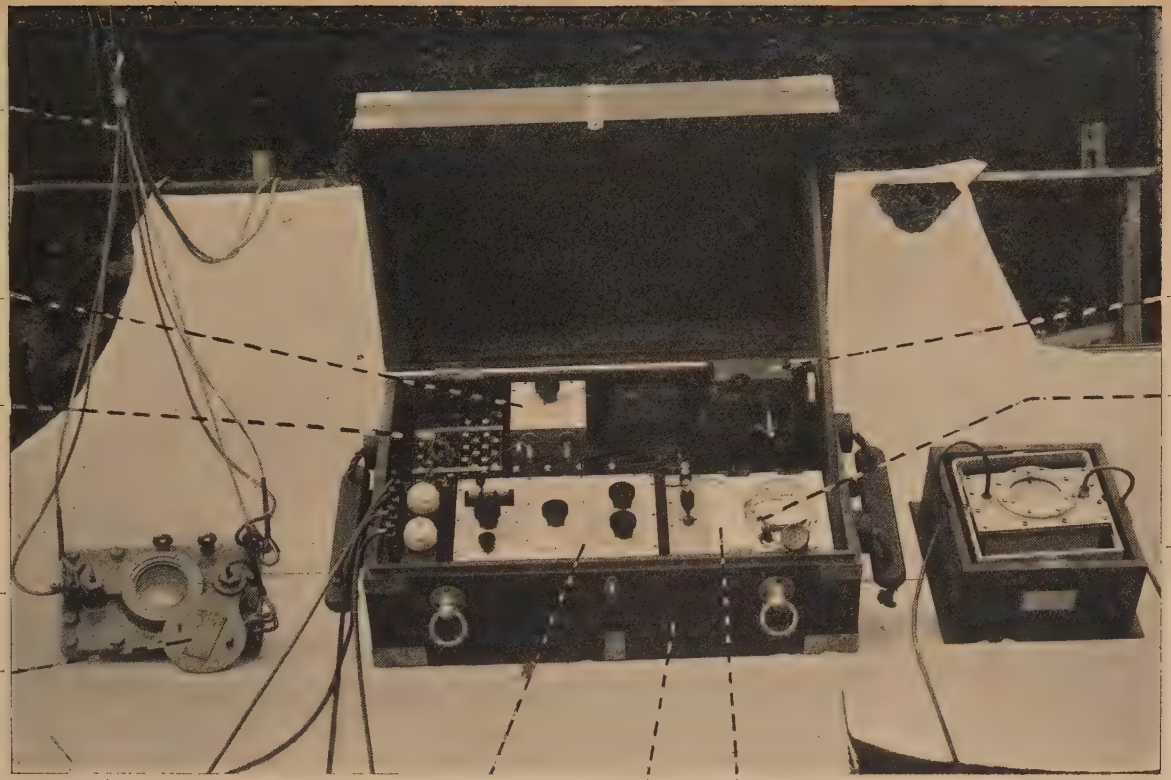
WIRE SLING.
STANDARD HIGH RESISTANCE 100,000 OHMS.
H.T. BATTERIES FOR PHOTO CELLS.
SEA PHOTOMETER.
SHUTTER OPEN.
CLOCK WORK INTERRUPTER.
TELEPHONES.
DECK PHOTOMETER ON GIMBALS.
SHUTTER.
CABLES TO PHOTOMETERS.
POTENTIOMETER.
SEA CHEST. METAL LINED.
AMPLIFIER.

FIG. 1.

the complete potentiometer and accessories. The instruments have no direct connexion between their metal cases, but are all mounted upon the common metal lining, so that all leakage must be localised to this. By raising the potential of any one of the cases to an appropriate value, any tendency to leakage can be neutralised. When finally tested the insulation was so perfect that it was found unnecessary to make use of the guard circuit facilities, but during fog and other bad conditions need might arise and they would then be available. The insulation of the finished apparatus was tested in a steam-saturated chamber for half an hour without any observable effect upon the insulation.

The potentiometer sea-chest contains all the apparatus required for the measurements. This consists of the high-tension battery, potentiometer, high resistance, standards cells, accumulator for the potentiometer, clockwork interrupter, and valve amplifier and telephone which takes the place of a very sensitive galvonometer in this equipment. Electric heating of the chest is provided for drying out in bad weather. This is intended for use with the ship's electric supply current, 110 volts D.C.

In the original instrument the clockwork interrupter was made of "Meccano" parts at very small cost. Although this has given wonderfully reliable service it was felt that something better should be provided in a finished design. A Morse telegraph clockwork movement was chosen on account of the known reliability of these for continual service. The interrupter is constructed upon fundamentally the same principles as that described by Poole and Atkins (1928), and consists of a ratchet-toothed wheel in rolling contact with a smooth jockey wheel. Mechanical refinements in adjustment for wear are introduced, and manganin is employed to reduce thermo-electric effects in the contact itself as these appear directly in the measuring circuit. Parasitic E.M.F.'s in the contact maker are further reduced by shunting a small condenser across the contact. The complete explanation of these parasitic E.M.F.'s is not fully clear, but they have been eliminated by these means. With the high amplification employed in the valve amplifier (about 30 decibels) any stray potential entering into the circuit becomes of importance. The clockwork drive is necessary because a motor is too liable to give stray electrical effects. The chief sources of these in the present instrument were found to be thermo-electric and frictional electrical effects in the mechanical contact maker and induction from external lighting and power circuits. The former were overcome by the use of manganin against brass, and the condenser as mentioned above, while the latter were completely eliminated by metal screening the whole circuit. This was rendered easy by the all-metal construction adopted for all the components of the circuit. A certain amount of experiment was

necessary to find the best points at which to connect the screens, but the final results fully justify the precautions. A very perfect silence can be obtained in the telephones, and the photo-electric currents balance with ample precision. The amplifier has three stages and is operated by dry cells contained in its own metal case, so that no leakage paths are anywhere provided, and further, the screening is rendered complete. The telephone leads also are screened and arranged to be at "earth" potential, so that the balance is quite unaffected by the operator's body. This also guards against insulation troubles from this part of the circuit. The precaution has one drawback, in that it definitely fixes the potential of the operator, so that when the high-tension battery is accidentally touched the full 50 to 100 volts is felt. This causes some surprise to the unsuspecting, but is a necessary price to pay for perfection of balance.

The Photo-electric Photometers.

The deck photometer and the sea photometer, although considerably larger, are constructed upon lines identical with the originals at the Marine Biological Association Laboratory. The cells themselves were specially made by the General Electric Company at Wembley and are much larger than those in use at Plymouth. This necessitates much more massive construction for the gun-metal casting in which the sea cells are contained.

The general appearance of the deck and submarine photometers is shown in Figure 1. In the latter the photo-electric cell is supported resiliantly from the lid on highly insulating ebonite supports. The bottom casting is sealed to the lid with a rubber gasket between the two machined faces and clamped by massive bolts. The glass windows are 3·2 cm. thick, and sealed between rubber washers by means of a clamping ring. A close-fitting sliding shutter closes this window when necessary to measure the "dark currents." This shutter is operated by cords from the surface, suitable fairleads being provided on the lid and wire cable upon which the submarine photometer is suspended. The two heavily rubbered cables to the terminals of the photo-electric cell enter the lid through glands, arranged to contract conical rubber stoppers on to the rubber covering of the cables. This type of joint has been found to be perfectly satisfactory. Drying chambers are provided inside the photometers to remove any residual moisture. When assembled, the insulation at the ends of the cables was more than a million megohms.

One submarine photometer is provided with cable sufficient for measurements down to 200 metres and the other for depths of 100 metres. Each is arranged for vertical suspension as well as horizontal. For vertical suspension the single wire cable shown in the extreme left of Figure 1 is

undone and fixed through a shackle hole in a fin on the bottom of the case midway between the two shackle eyelet bolts on right of case.

In the case of the photometer for use down to 200 metres, the photo-electric cell is a gas-filled one, but for use down to 100 metres, and for the deck photometer, vacuum cells were used. All three were potassium cells of approximately 7·6 cm. diameter and aperture, as figured in Type B by Campbell and Ritchie (1929).

The deck cell is contained in a light metal case, arranged for gimbal mounting, the gimbal ring remaining in a housing upon the deck, while the metal cell case can be removed to safety when not in use.

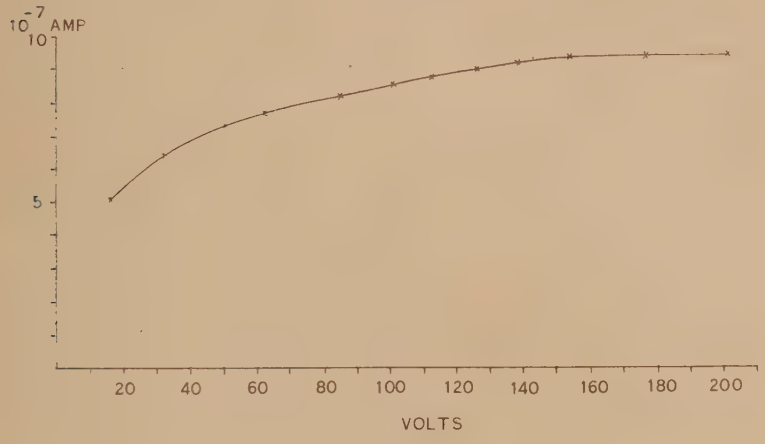

FIG. 2.

Over the window of the photo-electric cells discs of double surface flashed opalised glass are placed. These are held in position by three screw clips. It was found that owing to the great sensitivity of the cells, it is necessary at times to make use of diaphragms. These are of brass, 0·8 mm. thick, with central apertures 3·8 and 0·6 cm. in diameter. Figure 2 shows the voltage sensitivity curve for the vacuum cell housed in submarine photometer No. 1. From this it may be seen that at 140 to 150 volts saturation has been obtained. It is, however, quite permissible to operate the cell at 60 volts, at which point the sensitivity change amounts only to 0·33 per cent per volt. It seems safe to assume that the voltage sensitivity coefficient for the similar vacuum cell housed in the deck photometer is approximately the same.

Figure 3 shows the voltage sensitivity curve for the gas-filled cell housed in submarine photometer No. 2. The curve on the right was obtained with the large aperture diaphragm in position. The curve on the left was obtained subsequently without diaphragm. The point

marked F shows the reading obtained with the diaphragm in position at 16 volts after the first curve had been obtained. The sensitivity has much increased since the cell had been raised to a high voltage. For this reason in practice measurements are made after the cell has been caused to glow, by momentarily connecting it with a high voltage. In this cell the necessary voltage is 201 in the absence of light. It is inadvisable to work

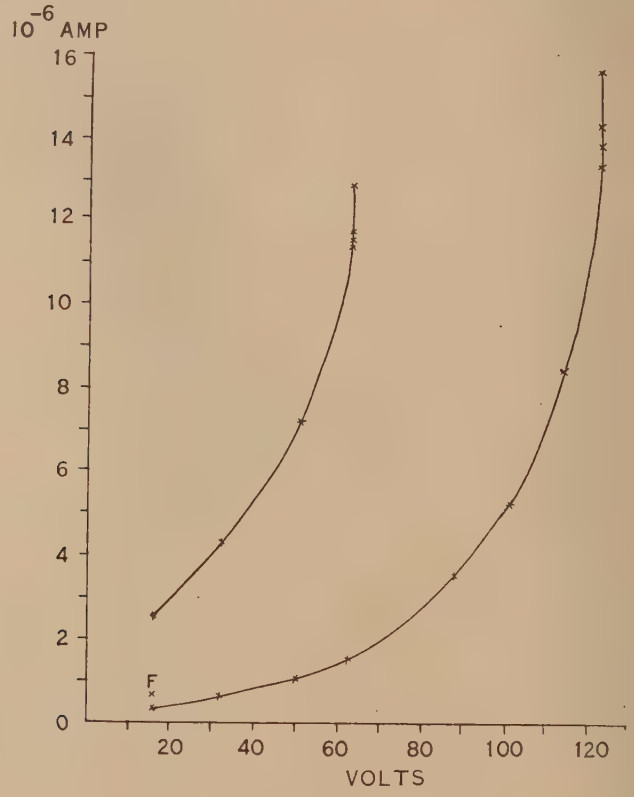

FIG. 3.

this cell under conditions leading to the production of a current greater than 50 μa (micro-amperes) owing to the steep rise in the curve at this reading. The deck photometer was standardised in diffused daylight against a similar vacuum potassium cell, which had been standardised by Poole and Atkins against an open carbon arc lamp (1928). This photometer had been in use for several years and was tested with a special lamp from time to time. It was shown that it had changed only slightly, if at all. The sensitivity is such that a current of 10^{-9} ampere is produced by a

vertical illumination of 40 metre candles or 42·4 for uniformly diffused light. When compared in diffused daylight, the new deck photometer was found to be 109 times as sensitive as the standard. Accordingly the *Atlantis* photometer at 60 volts anode potential gives one micro-ampere for 370 m.c. vertical illumination, or with uniformly diffused light for an illumination of 390 metre candles. The diaphragm reduces the illumination approximately to one-sixth, actually it is reduced 5·95 times. Consequently with diaphragm in position the vertical illumination necessary to produce one micro-ampere is 2200 metre candles vertical illumination or 2320 m.c. for diffuse light.

The No. 1 vacuum sea photometer at 60 volts anode potential requires 870 metre candles to produce one μa, or with uniformly diffused light 920 m.c. With diaphragm in position the values are respectively 5300 and 5600 metre candles. The gas-filled cell must not be regarded as quite constant in its behaviour, but the values given are those obtained immediately after the instantaneous glow when it is in its most constant and highly sensitive condition, according to the Research Staff of the General Electric Company (see also Atkins, 1931). With diaphragm in position and at 63 volts anode potential the gas-filled cell requires 1380 metre candles to give one μa, or 900 m.c. vertical illumination at 85 volts.

Without diaphragm at 63 volts 232 metre candles produce one μa, and at 85 volts 151 metre candles give one μa. It may be seen therefore that under these conditions the cell will measure down to less than one-sixth of a metre candle. For low illuminations the voltage may be increased very considerably and still smaller illuminations can then be measured.

The Neon Discharge Tube Photometer.

In addition to the potentiometer for measuring the photo-electric currents, one of Dr. J. H. J. Poole's neon lamp devices was provided. This is an extremely convenient means of measuring photo-electric currents, by counting the flashes of the neon lamp, the speed of which depends upon the amount of current flowing into a condenser, and therefore upon the rate at which the condenser builds up to sufficient voltage to discharge through the neon lamp. This instrument is illustrated in Figure 4. Its extreme simplicity is very attractive, and its application may be extended much beyond the present purpose. One feature worthy of note is that a given number of flashes can be regarded as proportional to a definite quantity of light, irrespective of the time taken. Thus the photographer can calibrate any emulsion in terms of the number of flashes necessary to give correct exposure, irrespective of the intensity of illumination.

The neon discharge tube photometer was assembled exactly as figured

by J. H. J. and H. H. Poole (1930), with the exception that the paraffin wax was extended to cover all the internal wiring in the housing instead of being placed around the neon discharge tube only. The petticoat type of terminal was used on the outer side of the casing, as shown in Figure 4.

The apparatus contains four condensers of capacity 0·5 to 0·0005 μF. Even with 0·5 μF. the current given by the highly sensitive photo-electric cells supplied with this outfit was so great that the flashing in bright daylight was too rapid to count, even with the diaphragm necessary for use with the potentiometer apparatus. Accordingly a smaller

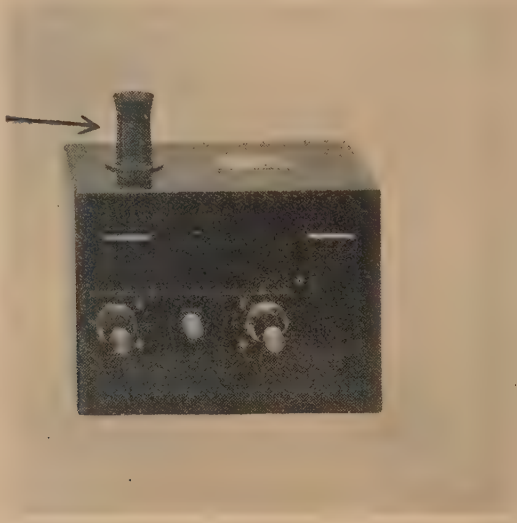

Fig. 4.

diaphragm, 0·6 cm. aperture, was obtained for use with the neon discharge tube.

The time before the ship sailed was too short to permit of a complete standardisation of the sensitivity of the neon discharge tube photometer with the various condensers. It was, however, ascertained that when one of the photo-electric cells was almost closed and placed in diffused light in a room so that it gave a current of 30×10^{-9} ampere, the neon lamp just maintained a slow rate of flash. Its sensitivity, therefore, appears to be ample for use down to a considerable depth, though its sphere of usefulness is most pronounced in that region, immediately under the surface and at small depths, at which the light varies very rapidly owing to the motion of the waves.

The high-tension batteries for use with the neon discharge tube are

housed in a suitable box with a small volt-meter attached to its lid. Flashing in the lamp began at a voltage of 236 and did not take place at lower voltages however great the illumination. For use it is advisable to have a voltage considerably—say, about 60 volts—above this, so that there may remain an adequate voltage for the working of the photo-electric cell at all times in the cycle of charge and discharge of the tube. The whole apparatus was taken on board the *Atlantis* and a trial was made at sea in the neighbourhood of the Eddystone Lighthouse, off Plymouth. It was not possible to test the submarine photometers down to a depth of more than about 40 metres, but at such depth they showed no sign of leakage.

As regards the depth to which it is possible to measure illumination, it may be said the gas-filled cell is provided with cable to go to 200 metres. The illumination at that depth is probably quite measurable though small. Poole and Atkins (1929) arrived at an approximate formula connecting the depth at which it was possible to see the Secchi disc and the vertical absorbtion coefficient. The latter is roughly equal to 1·7 divided by the depth in metres. It is on record that in the open ocean the disc has been seen to as great a depth as 66 metres. From this a vertical absorbtion coefficient 0·026 is derived ; such a value would lead one to expect a quite measurable amount of light at 200 metres. The results obtained on board the *Atlantis* are, however, to be awaited with great interest. They should offer an interesting comparison with visual observations made by Beebe in the bathysphere.

All the apparatus described in this paper was manufactured and assembled at the works of Messrs. H. Tinsley and Company, except the photo-electric cells and neon discharge tube, which were supplied by the General Electric Company, Wembley, Middlesex.

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Abstracts of Memoirs

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY.

The Photo-electric Recording of Daylight.

By W. R. G. Atkins and H. H. Poole.

Nature, 1930, **125**, 305.

A Burt vacuum sodium photo-electric cell was mounted in a heavy gun-metal case, such as used in submarine photometry. A sheet of opal glass was placed above the window. This acts as an efficient diffusing surface and is set horizontally so as to measure vertical illumination. High-tension ignition cables lead from this to the laboratory where the high-tension batteries are stored together with a Cambridge Instrument Co. " thread-recorder." In this manner continuous records of daylight have been obtained.

W. R. G. A.

Some Geochemical Applications of Measurements of Hydrogen Ion Concentration.

By W. R. G. Atkins.

Sci. Proc. Roy. Dublin Soc., 1930, **19**, 455–460.

The reaction of natural waters may vary between pH 1·5 and pH 10·0. Obviously the reaction has an important influence upon the solution of rock and upon precipitation of metallic hydroxides already in solution. It is found that ferric salts are precipitated when the solution is still markedly acid. Ferrous salts remain in solution at a much lesser degree of acidity. The presence of organic matter, owing to its reducing action, favours the solution of iron ore. The problem of the formation of dolomite may also be regarded from this point of view. There are certain marked differences between the behaviour of the carbonates, bicarbonates and hydroxides of calcium and magnesium. The weathering of basalt has also been studied. Strongly weathered basalt is acid in reaction. Basalt which has only been slightly weathered gives a reaction which is neutral or only slightly acid, whereas a freshly exposed basaltic surface may be quite alkaline. It is obvious that considerations such as these must enter into a large number of geological problems.

W. R. G. A.

On the Photo-electric Measurement of Daylight.

By W. R. G. Atkins and H. H. Poole.

In the Photo-electric Cells and their Applications, published by the Physical and Optical Societies. Discussion on June 4th and 5th, 1930, 128–137.

Previous work on daylight and submarine photometry is reviewed. Details are given of the standardisation of the photo-electric photometers against a carbon arc. Measurements are described with the General Electric Co. new cæsium monatomic vacuum cell, type CMV 6. This is sensitive throughout the whole spectrum. A method was evolved for comparing light of different colours by obtaining the ratio of the blue to red, blue to yellow and blue to green readings, using colour filters. These are obtained in white light, and again in light of different composition. The method has been described at greater length in a subsequent paper.

W. R. G. A.

Photo-electric Measurements of Illumination in Relation to Plant Distribution. Part 3. Certain Spruce, Larch, Oak and Holm Oak Woods.

By W. R. G. Atkins and Florence A. Stanbury.

Sci. Proc. Roy. Dublin Soc., 1930, **19**, 517–531.

A portable photo-electric outfit with micro-ammeters for measuring current has been found serviceable over rough ground. Under shade from *Picea excelsa* the daylight factor was reduced to 1–2 per cent. There was little or no growth on the ground save occasional seedlings of *Hedera helix*. Under shade from a narrow wood of *Larix europæa* the value of the daylight factor was about 15 per cent. There was a good undergrowth of *Rubus fruticosus*, a belt of *Pteris aquilinum* and a woodland flora.

In a wood of *Quercus ilex* on level ground the daylight factor was found to be 1–2 or 3 per cent. In many sites there were pure carpets of Hedera. The deepest shade found was under *Ilex aquifolium* where the daylight factor was only 0·6 per cent.

W. R. G. A.

The Distribution of Pasture Plants in Relation to Soil Acidity and other Factors.

By W. R. G. Atkins and E. Wyllie Fenton.

Sci. Proc. Roy. Dublin Soc., 1930, **19**, 533–547.

Many British grasses are distributed over pastures varying from the most alkaline to almost the most acid found. Some have a range which is limited on the acid side, viz. *Cynosurus cristatus*, *Lolium perenne* and

Dactylis glomerata, but *Nardus stricta*, *Aira flexuosa* and *A. cæspitosa* are only found on acid soil. Of leguminous plants *Ulex europæa* extends throughout the whole range. *Trifolium repens* is the most important leguminous constituent of acid pastures, in which, however, it is not found in soil more acid than pH 5. *Trifolium pratense* is rare beyond pH 6, as are most of the other leguminous plants. In a well-drained alkaline soil *Medicago sativa* was found to persist, when sown, for six years. It was found that when allowed to roam freely sheep and cows grazed closely on herbage growing in soil at pH 6 and on most sites less acid than pH 5; in more acid sites grazing did not take place, or if it did, only slightly. The use of a neutral solution of potassium chloride in making soil extracts leads to large errors through base exchange.

W. R. G. A.

Photo-electric Measurements of Illumination in Relation to Plant Distribution. Part 4. Changes in the Colour Composition of Daylight in the open and in shaded situations.

By W. R. G. Atkins and H. H. Poole.

Sci. Proc. Roy. Dublin Soc., 1931, **20**, 13–48.

Data are presented concerning the transmission of various colour filters. For work in shaded situations J. H. J. Poole's neon lamp device was found to be the most convenient measuring instrument. The cæsium vacuum cell CMV 6 was satisfactory for such work. Its infra-red sensitivity may be suppressed by a Corning heat-absorbing filter so that it gives a wave-length sensitivity curve which is approximately horizontal between 440 and 640 mμ. By means of the above cell with a diffusing opal glass and colour filters it is possible to obtain a measure of the colour of the light under different conditions and in woods, taking daylight of some definite type as standard. In woods the light is very poor in blue, relatively rich in green, is close to sunlight in orange-red, and very much richer than either skylight, daylight or sunlight in the deep red. The source of light in woods is predominantly blue sky or grey sky rather than normal daylight with sunshine. Measurements have been made of the relative intensities of direct sunlight and diffuse skylight. Sunlight being relatively much richer in the light towards the red end of the spectrum, the value of this ratio at any given time depends on the type of photo-electric cell used to measure it, and is greatly altered by the use of colour filters.

W. R. G. A.

Observations on the Photo-electric Measurement of the Radiation from Mercury Vapour Lamps and from the Sun, and on the effects of such Radiation upon the Skin.

By W. R. G. Atkins.

Sci. Proc. Roy. Dublin Soc., 1931, **20**, 49–65.

A glass vacuum sodium photo-electric cell was used with a micro-ammeter to show that it is advisable to run a mercury vapour arc continuously, exposure being made six minutes after switching on. After thirty-five minutes it should be switched off to allow of cooling. Five minutes later its initial potency has been recovered ; the loss due to overheating is a real phenomenon. The aluminium reflector used, when adjusted to its position of maximum efficiency, increases the total radiation about 2·7 times and the ultra-violet radiation about 2·0. This more than counter-balances any loss of efficiency in the lamp due to overheating occasioned by the reflector. When the reflector is used the decrease in intensity is considerably less than that calculated according to the inverse square law. J. H. J. Poole's neon lamp is very convenient for the study of the mercury vapour arc. Data are given concerning the times required for the arc to produce slight erythema. These times are compared with those required for sunlight of different intensities reckoned in thousand metre candles. Approximate photo-electric determinations of the ratio of total to diffuse ultra-violet show that even at midsummer noon the sunlight is not very much more potent than the diffuse light from the whole sky and the direct light must be relatively less intense at other times.

W. R. G. A.

Some Experiments on the Accuracy obtainable with Gas-filled Photo electric Cells.

By W. R. G. Atkins.

Sci. Proc. Roy. Dublin Soc., 1931, **20**, 67–73.

Gas-filled cells were tested for constancy of emission immediately after the glow-discharge had been passed momentarily. The cæsium hydride cell behaved irregularly. The potassium hydride cell is more constant, and readings could be obtained to within 2 per cent with anode potential 60 volts. No tests were made as to the constancy or otherwise of the cell over prolonged periods. The rate of decrease of sensitivity after the glow amounts to about 2 per cent per minute. It is therefore advisable to make measurements immediately after the discharge which should be of momentary duration only. From this it follows that, if Poole's neon lamp method be used to integrate the current, such integration should be over equal periods of time in each case rather than for an equal number of flashes of the lamp.

W. R. G. A.

The Penetration of Light through successive Layers of Tissue-paper.

By W. R. G. Atkins.

Nature, 1931, **128**, 545.

A sodium photo-electric cell was used to measure the light transmitted and it was found, when allowance has been made for the extra loss at the first surface, that the succeeding layers from numbers 2 to 10 transmitted fairly regularly, the transmission factor being 79 per cent. It was found that 8·4 per cent of the original light remained after passing through ten layers of paper. The results bear on previous letters to *Nature*.

W. R. G. A.

The Treatment of Gapes in Chicken.

By W. R. G. Atkins.

Nature, 1931, **128**, 585.

Between February and May fourteen chicken affected with gapes were treated with a dilute solution of carbon tetrachloride in medicinal paraffin. Only one died, whereas all untreated chicken died in the same period. The chicken were about one month or six weeks old. At the end of May, however, the treatment broke down completely when applied to chicken only a fortnight old. None of these recovered. One, however, never contracted gapes though freely exposed. The treatment appears to be worth careful investigation.

W. R. G. A.

The Adductor Mechanism of Pecten.

By L. E. Bayliss, E. Boyland and A. D. Ritchie.

Proc. Roy. Soc., 1930, *B.*, *Vol.* 106, *p.* 363.

A study has been made of physiological behaviour of the adductor muscle of Pecten. The slow part of this muscle can be isolated from the nervous system in a completely relaxed state in *P. magellanicus*. Stimulated electrically, it gives twitches about 100 times as slow as frog's skeletal muscle, which can be fused to form a tetanus. The difference in time scale is determined by the viscosity of the tissue, which in this muscle is about the same in the excited and unexcited states. The tensions developed are large. In *P. maximus* and *P. opercularis*, on the other hand, different nervous connections make it difficult to isolate the muscle without some "contracture"; apart from this, however, the muscles of these animals behave in the same manner as those of *P. magellanicus*.

The state of " contracture " is a result of reflex excitation which survives isolation, but may be partly or completely abolished by direct faradic stimulation. The " contracture " is not accompanied by increased viscosity, and it is uncertain whether it results from a continued state of excitation; the tensions in " contracture " are much less than the maximum tensions obtainable. Reflex movements of intact animals suggest that for the most part the muscle is contracting tetanically and is not in a state of " contracture."

The quick muscle gives a rapid twitch with single induction shocks that resemble the normal reflex contraction (swimming movement or flap). Complete fusion of twitches is not readily obtained, and the maximum number of contractions obtained by stimulation is not large, although varying in different species according to the animal's normal activity.

L. E. B.

Studies in Tunicate Development. Part I. General Physiology of Simple Ascidians.

By N. J. Berrill.

Phil. Trans. Roy. Soc. B., Vol. 218, 1929, *pp.* 37–78.

The following species were investigated: *Tethyum pyriforme*, *Boltenia hirsuta*, *Styelopsis grossularia*, *Phallusia mammillata*, *Ascidiella aspersa*, *A. scabra*, *Ascidia conchilega*, *A. mentula*, *A. prunum*, and *Ciona intestinalis*.

In the egg the perivitelline space is due to colloids exerting an osmotic pressure equivalent to 0·8 per cent gum-arabic in sea-water. In the oviduct this is counterbalanced by substances in the oviducal fluid. " Organ-forming " substances similar to those in the egg of *Styela partita* but much more striking occur in the egg of *Boltenia hirsuta*.

In the Ascidiidæ, Cionidæ, and probably in all ascidians with small eggs, the tadpole larva hatches through the digestion of the egg-membrane by a proteolytic enzyme active within the limits pH7–10. An alternative method which is only possible later in development is by rupture of the membrane, usually by ectodermal ampullæ. These ampullæ combine the functions of respiration and fixation.

The onset and rapidity of metamorphosis may be controlled by varying the hydrogen-ion concentration of the water. Hyper-alkalinity tends to inhibit metamorphosis, in particular tail-absorption, altogether; increased acidity tends to induce metamorphosis. The total salt concentration of sea-water has little effect upon development other than that of retardation.

The great variability of eggs and larvæ is due primarily to the length of time an egg has lain within the oviduct and to the degree of toxicity of the ovidical fluid during that time.

N. J. B.

Studies in Tunicate Development. Part. II. Abbreviation of Development in the Molgulidæ.

By N. J. Berrill.

Phil. Trans. Roy. Soc. B., Vol. 219, 1931, *pp.* 281–346.

The development of 16 species of Molgula and 2 of Eugyra is described. Ten are viviparous. Viviparity is due to reduction in adult size (in this family) in turn due to precocious sexual maturity resulting from the colonisation of exposed rocks by sand-adapted types. Increase in egg-size is a result of viviparity. Nine species develop without forming tailed tadpoles. Two species said to be anural were found to produce tadpoles. Cleavage is identical in anural and urodele development. Hatching in anural development is always by membrane rupture and never by digestion. An aggregation of cells is always seen near the larval stomach in both types of development; in the urodele it is known to be the absorbed tail. Failure of tail development is due to the absence of swelling on the part of the notochord cells, which, however, are present in normal number. Failure to extend on the part of the notochord results in a delay in closure of the blastopore. Imbibition by notochord cells is to some extent dependent upon the alkalinity or acidity of the surrounding medium.

Out of 139 species of the Ascidiacea anural development is confined to 9 molgulids. Within the Molgulidæ the origin of anural development is polyphyletic. The otolith (the only sense organ) is present in all urodele larvæ, absent in all anural larvæ. Anural development is to be correlated with large size, oviparity, and a free, unattached sand life; urodele more with small, viviparous, attached forms. The first type is considered to be primitive, and to have secondarily colonised the rocks.

It is concluded that anural development is a direct response to the sand-embedded habitat, that the hatching enzyme is lost when tail development is disturbed, and that anural development is compatible with race-survival only when there is an efficient alternative method of hatching.

N. J. B.

Regeneration in *Sabella pavonina* (Sav.) and other Sabellid Worms.

By N. J. Berrill.

Journ. Exp. Zool., Vol. LVIII, 1931, *pp.* 405–523.

The account represents a preliminary investigation into the phenomena of regeneration and metamorphosis typical of this group of annelids. In a normal worm the body is divided into three regions, a head and collar, a thoracic, and an abdominal region. The thoracic and abdominal segments differ in that the setæ and uncini in the first are respectively ventral and

dorsal, while in the second they are dorsal and ventral, as though the anterior part of the worm is twisted through 180 degrees. In development thoracic segments are first formed and then abdominal segments added behind.

In regeneration from anterior cut-surfaces, only a head and collar segment is formed from any segment of the body; from posterior cut surfaces regeneration is continuous as in normal growth. Up to two or three thoracic segments can be formed from the posterior surface of thoracic segments. In isolated abdominal pieces thoracic segments are always formed through the metamorphosis of originally abdominal segments. The usual number is 5–12, but under certain imperfectly known conditions it may be from 0–80. Metamorphosis never extends into abdominal segments newly regenerated posteriorly. There is always a gradient in degree of metamorphosis, the process being completed anteriorly while but just starting posteriorly. Contact with thoracic structure is unnecessary for abdominal metamorphosis, and every abdominal segment in the body is potentially thoracic. Temperature has merely an indirect influence, actual and physiological age being of greater importance in determining the extent of transformation. The reorganizing stimulus resides in the anterior regenerated head and collar segments. A hormonal control is precluded, while there is some evidence that it may be neuroid or electrical.

N. J. B.

Untersuchungen zur Physiologie der Tonusmuskeln.

By E. Bozler.

Zeitschr. f. vergl. Physiol. 12, *pp.* 579–602.

Two preparations of nonstriated muscle, suitable for quantitative experiments, are described, the retractor of the pharynx of the edible snail and the nonstriated part of the adductor muscle of Pecten. They respond easily to electrical stimuli, also to weak single induction shocks. The isometric twitch is very asymmetric, the rise of tension is as rapid as in most striated muscles, the relaxation is relatively slow. The resting muscle behaves like a plastic body; stretching produces tension. This, however, disappears completely, if the length remains constant. The time relations of this process are exactly the same as those of relaxation in an isometric twitch. In the Pecten muscle the quickness of the relaxation may change within wide limits in the same muscle preparation. After stimulating with a series of shocks, the muscle relaxes more quickly than if single shocks be applied in long intervals. In the discussion of the nature of this change it is important that the mechanical properties

of the muscle change at the same time in a definite manner. This change seems to be of great importance for the energetics of the muscle and explains some of the peculiarities of nonstriated muscle. E. B.

Notes on the Hydrogen Ion Concentration Excess Base and Carbon Dioxide Pressure of Marine Aquarium Waters.

By E. M. Brown.

Proc. Zool. Soc., 1929, *Pt.* 4, *pp.* 601–613.

A comparison of tank-water from the Aquarium of the Zoological Society of London with that of Plymouth showed that, while both deviate from the normal in certain respects, the former shows a greater variation in different tanks and at different times.

Plymouth maintains a nearly constant pH, as a result of liming, but is supersaturated with carbon dioxide. The Zoo Aquarium water shows a relatively low varying pH, low excess base and high carbon dioxide pressure, but a large variety of fish and invertebrates adapt themselves successfully to these conditions.

The diminution of the excess base in untreated Aquarium water was examined and the advantages of liming were discussed. E. M. B.

The Action of Potassium Cyanide and Potassium Ferricyanide on certain Respiratory Pigments.

By S. F. Cook.

Journ. Gen. Physiol., Vol. XI, No. 4, 1928.

Measurements with the Barcroft differential manometer show that different types of respiratory pigments are affected differently by $K_3Fe(CN_6)$ and KCN. The oxygen in hemoglobin is liberated by the former and not the latter, that in hemocyanin by the latter and not the former, that in echinochrome by the former and not the latter, and that in hemerythrin by both. S. F. C.

The Anatomy and the Histology of Bud-Formation in the Serpulid *Filograna implexa*, together with some Cytological Observations on the Nuclei of the Neoblasts.

By G. H. Faulkner.

Journ. Linn. Soc. Zool., Vol. XXXVII, 1930, *pp.* 109–190.

In the anatomy of *Filograna implexa* the chief points of interest are: the nephridia and their homology with other Polychaet nephridia: the

nerve supply to the branchial filaments : the circulatory system and the support this gives to the theory of a blastocoelic origin of the Annelid blood spaces.

During summer Filograna reproduces sexually, and during spring asexually, by a process of transverse fission. Posterior to the future plane of fission a new anterior region consisting of a head and two setigerous segments is inserted, and anterior to it a new growing point for the stock. The position of the abdomen of the plane of fission is variable.

The segments which are destined to be separated as the bud undergo prior to fission a complete histolysis. The old tissues break down, partly independently and partly by phagocytosis and are replaced by new embryonic tissues. A similar rejuvenation of tissues occurs to some extent on the stock. The cells which lay down the new tissue arise by the proliferation of neoblasts in the ventral body wall. These neoblasts are identical with the definitive germ cells of the sexual season. They are distinguishable from other cells by the fact that during resting stages their chromosomes persist as condensed bodies lying in pairs in a clear nuclear cavity.

G. H. F.

A Spectrographic Analysis of Animal Tissues.

By H. M. Fox and H. Ramage.

Proc. Roy. Soc., B., Vol. 108, 1931.

The present paper is the first report on a quantitative spectrographic analysis of animal tissues. It deals largely, but not exclusively, with annelids and molluscs. Iron and copper were present in all tissues analysed. Manganese was widely distributed. The manganese content of tissues varies with locality. Its function is discussed. Nickel and cobalt occurred spasmodically, the former being more frequent. Except in one case, all high concentrations of nickel were accompanied by cobalt. In one tissue only did cobalt occur without detectable nickel. Lead and silver both exhibit an irregular distribution. Certain tissues have a disposition to accumulate one or other of these elements. One man had silver in all organs, two others had none. Lead accumulates in different tissues of different individual men. Cadmium occurred in the livers of *Pecten maximus* from different situations. Lithium is very widespread in animal tissues, rubidium is much less so, while cæsium was not found. Strontium was found in numerous cases. Barium was not detected. Calcium fluoride was found in one case only, namely, the body-wall of *Archidoris tuberculata*.

H. M. F. AND H. R.

Notes on Protodrilus.

By E. S. Goodrich.

Quart. Journ. Micr. Sci., Vol. LXXIV, 1931, *pp.* 303–319.

The nephridium of *Protodrilus flavocapitatus* is described in detail. With its long-coiled canal and small projecting nephridiostome it is shown to be more complicated than hitherto supposed.

The sperm-duct of the male has a ridged ciliated cœlomostome and represents a cœlomoduct or possibly a nephromixium. It is argued that the "brachynephridia" and sperm-ducts of all Protodrilids are of the same morphological nature.

The fate of the cœlomoduct is related to the mode of emission of the genital products. In the female of *Pr. flavocapitatus*, which sheds the ripe ova by dropping off posterior segments, the cœlomoducts have been lost in all the segments, and in the male in all the segments excepting the eleventh. Remains of the cœlomostomes are perhaps represented by the ciliation of the cœlomic epithelium in the genital segments of the female.

It is maintained that Protodrilus is dicœcious, that the female may be early inseminated by the male, that copulation must take place, and that the dorsal glands are perhaps concerned in the process. Ripe spermatozoa only are found in the female, and the so-called stages in "cystospermatogenesis" are probably stages in the phagocytosis of superfluous spermatozoa.

E. S. G.

On the Morphology, Feeding Mechanisms, and Digestion of *Ensis siliqua* (Schumacher).

By A. Graham.

Trans. Roy. Soc. Edinburgh, Vol. LVI, pp. 725–752, 1931.

The structure of the razor shellfish *Ensis siliqua* is briefly described. A description of the byssus gland, which is only found in very young specimens, is given, while the structure of the nervous system is described in detail. The four principal ganglion pairs of the Lamellibranch are present, the cerebral and pleural ganglia on each side being fused, while the two pedal ganglia have fused to form a median ganglion; the two visceral ganglia lie closely approximated. The peripheral portions of the nervous system are remarkable for the large number of pallial anastomoses, there being four main circumpallial loops in each mantle fold in addition to minor connections. An asymmetrical stomatogastric system

supplying the anterior parts of the alimentary canal and in connection with vestiges of the buccal ganglia, is represented.

An account is given of the pallial ciliary currents : these appear to be in essentials similar to those described for other genera, save that the rejection tracts on the mantle are more powerful. The mode of functioning of the selective mechanisms on the palps is discussed.

The place of secretion of the style is described as two longitudinal tracts in the style sac of granular, darkly staining (iron hæmatoxylin) cells ; these are held to be homologous with the major and minor typhlosoles of those forms where the style sac and the mid gut are confluent.

The style diastase has an optimum temperature of 35° C. at pH 6·0 and an optimum pH of 6·0 at 22° C. The style also contains an oxidase.

The protease of the digestive diverticula has an optimum temperature of 32° C. and optimum pH values of 4·2 and 8·2 at 30° C. The diverticula also contain a lipase, a diastase and a glycogenase.

A. G.

On the Optimum Hydrogen Ion Concentration and Temperature of the Style Enzyme of *Pecten maximus*.

By A. Graham.

Proc. Roy. Soc., B., Vol. 108, *pp.* 84–95, 1931.

The diastase of the crystalline style of *Pecten maximus* was studied in order to investigate :—

(*a*) The effect of time on the pH optimum.
(*b*) The effect of pH on the temperature optimum.
(*c*) The effect of time and temperature on the pH optimum.

The principal results were as follows :—

(*a*) There is no variation in the pH optimum with variation in the duration of the experiment.

(*b*) There is a fall in the optimum temperature accompanying a fall in the pH of the medium.

(*c*) When two out of the three factors under the control of the animal —time, pH, and temperature—are made equal to those found in natural conditions, and the optimum for the third is determined, it also is found to be the actual condition encountered in the living animal.

A. G.

On the Correlation of the Life-history of the Acephaline Gregarine, Gonospora, with the Sexual Cycle of its Host.

II. *Gonospora* (*Kalpidorhynchus*) *arenicolae*.

By C. C. Hentschel.

Parasitology, XXII, 4, 505–509, *November*, 1930.

It is shown that there is a correlation between the life-history of the acephaline gregarine, *Gonospora arenicolae*, and the sexual cycle of its host, *Arenicola ecaudata*. This correlation exhibits certain points of similarity with that between the allied species *G. varia* and its host *Audouinia* (*Cirratulus*) *tentaculata*, described in a previous paper. The correlation is, however, not so definite, for the life-history of a generation of parasites does not necessarily coincide with the sexual cycle of the host. The ejection of the sporocysts together with the worm's gametes was observed. A brief description of the spawning behaviour of *Arenicola ecaudata* is also given.

C. C. H.

Regeneration of the Spines in Sea-Urchins.

By A. D. Hobson.

Nature, Vol. 125, *p.* 168, 1930.

Specimens of *Psammechinus miliaris* exposed to direct sunlight in the laboratory threw off all their spines except those on the oral surface. Regenerated spines were visible in a week and were fully developed in two months.

A. D. H.

Carbohydrates of Crab Nerve.

By E. G. Holmes.

Biochem. Journ., Vol. XXIII, 1929, pp. 1182–1186.

The peripheral nerves and nerve ganglia of Maia and Cancer are extremely rich in carbohydrate, which is present as glycogen and as "free carbohydrate." In the case of the ganglia, some, at least, of the carbohydrate is present as di- or poly-saccharide, soluble in 60 per cent alcohol, and in Schenk's reagent. In nitrogen there is hydrolysis of glycogen, and in the case of the ganglion, of the soluble di- or poly-saccharide ; there is also formation of lactic acid. In oxygen, the formation of lactic acid is inhibited ; the breakdown of glycogen is less than occurs in nitrogen.

E. G. H.

Observations on Dicystid Gregarines from Marine Worms.

By D. L. Mackinnon and H. N. Ray.

Q.J.M.S., Vol. LXXIV, 1931, *pp.* 439–466.

The detailed classification of dicystid gregarines is in a very confused state, and some attempt was made to correlate and correct the observations by earlier workers.

Dicystids in polychæte worms were studied, and notes made on the structure of two species of Polyrhabdina, occurring in *Scololepis fuliginosa* and in *Polydora flava* respectively.

Two new dicystids, for which the generic names Hentschelis and Lecythion were proposed by us, were described from the intestine of the echiurid worm, *Thalassema neptuni*. Spore-formation, after evacuation of the associated gametocytes into sea-water, was observed, and flagellated male gametes were seen to develop; but it was not possible to decide certainly to which species of gregarine in Thalassema these should be referred.

D. L. M.

A New Protozoon, *Hyperidion thalassemae* n. gen., n. sp., from the Intestine of *Thalassema neptuni* Gaertner.

By D. L. Mackinnon and H. N. Ray.

Q.J.M.S., Vol. LXXIV, 1931, *pp.* 467–475.

A new sporozoan, for which we propose the name *Hyperidion thalassemae*, was observed in the gut of *Thalassema neptuni*. This is an acephaline, pestle-shaped gregarine, which lives within modified epithelial cells while it is young; but which later on protrudes a contractile, club-like portion of its body into the lumen of the intestine. Its possible relationship with *Zygosoma gibbosum* (Greeff) from *Echiurus pallassii* were considered.

D. L. M.

The Feeding Mechanism, Formation of the Tube and Physiology of Digestion in *Sabella pavonina*.

By E. A. T. Nicol.

Trans. Roy. Soc. Edin. III, LVI, pp. 537–598, 1930.

The branchial crown of *Sabella pavonina* is a specialised ciliary filtering organ which collects small particles of detritus, diatoms, flagellates and similar organisms, as well as fine sand, from the water. The ciliation of the pinnules causes water to flow between the filaments into the branchial funnel. Particles in suspension are caught by cilia and carried down the filaments to the basal folds, where they are sorted into three grades, the

criterion being size not weight. The largest particles are rejected, the medium are stored in the ventral sacs (to be used later for tube building), while the smallest are carried to the mouth. The distribution of mucus cells on the branchial crown shows clearly that they are associated with the rejecting, and not with the collecting tracts of the ciliary apparatus.

The enzymes present in the gut are an amylase, protease and lipase. The optimum hydrogen ion concentrations at which these enzymes work are 6·8, 7·8, and 7·4 respectively, all of which fall within the limits of the hydrogen ion concentration of the gut, 6·0–8·4.

E. A. T. N.

Giant English Oysters.

By J. H. Orton.

Nature, Vol. CXXVI, August 30, 1930.

Details are given of size, weight and sex of two giant oysters, *O. edulis*, taken by E. A. T. Nicol and W. Searle near the Salstone, Salcombe Estuary, September 20th, 1929. It is of some economic importance that both the oysters were females, one mature which extruded a few ripe ova, and one maturing. Evidence is thus obtained that old oysters breed as females. From the volume of the gonad in the larger specimen (the shell of which was 17·6 cm. long by 19·8 cm. broad and 5·9 cm. wide) it is estimated that this individual had the capacity to produce 10 to 20 cm. of eggs—the equivalent of three to six million larvae—in one batch; hence the value of such oysters for repopulating old or new beds may be inferred.

J. H. O.

On the Oyster Drills in the Essex Estuaries.

By J. H. Orton.

Essex Naturalist, Vol. XXII, 1930.

Three oyster drills are recorded from the oyster beds of the Essex Estuaries, namely, *Ocenebra erinacea* (L.), *Nucella* (=*Purpura*) *lapillus* (L.) and *Urosalpinx cinerea* (Say). Figures of the shells and egg-capsules of each species are shown and their habits discussed. Experiments are cited showing that Urosalpinx and Ocenebra eat small (brood) oysters in about three days, and larger (half-ware) oysters in five or six days. It is shown that the habits of Ocenebra and Nucella vary in different habitats. Nucella attacks oysters, but probably much less frequently than the two other tingles. The introduction of the American oyster tingle, Urosalpinx, into English waters is discussed; the earliest record of its occurrence is 1920, though the date of introduction is undoubtedly much earlier.

Incidentally a record is given of an observation on the introduction of living Crepidula and Anomia into English waters on American oysters in 1908. The importance of the suppression of the tingle pests for successful oyster-culture is emphasised, as well as the economic value of knowledge of the habits of these animals.

J. H. O.

Oysters and Oyster Culture.

By J. H. Orton.

Encyclopædia Britannica, 14th Edition, 1929.

The article consists of a much condensed account of oysters and oyster culture. The chief economic species are noted. The incubatory and external modes of development are described briefly, with paragraphs on food and mode of feeding, oyster beds, fattening, shell growth, enemies and parasites, diseases, the oyster industry, oyster culture and breeding. In the latter paragraph are brought together the records published to that date of the spawning seasons of *O. edulis* and *O. virginica*. The arrangement of the records in localities of increasing latitude, as follows, shows earlier spawning in the warmer situations.

Species.	*Locality.*	*Range of spawning period.*	*Reference.*
O. edulis	Taranto Gulf (Italy)	March – April to October	Proprietors (Dean)
	Arcachon (France)	May to September	De Bon
	Thames Estuary (England)	End of May to September	Anson and Willett
	Fal Estuary (England)	End of June to September–October	Orton
	Norway (fattening ponds)	August to September	Helland-Hansen
	Norway (offshore)	Little or rare	Do.
O. virginica	Chesapeake Bay* (Md.)	May – June to August	Winslow-Brooks
	Barnegat Bay (N.J.)	End of June to ?	J. Nelson
	Malpeque (Canada)	June–July to ?	Stafford

J. H. O.

* Recently Hopkins (*Bull. Bur. of Fisheries,* U.S. Dept. of Commerce, Bull. No. 3, 1931, Washington) records spawning in Galveston Bay (Gulf of Mexico) from end of March to at least August in 1929.—J. H. O.

The Adaptation of *Gunda ulvae* to Salinity. I. The Environment.

By C. F. A. Pantin.

Journ. Exp. Biol., Vol. VIII, 1931, *pp.* 63–72.

The environment of the triclad *Gunda ulvæ* has been studied. This organism lives on the seashore in the estuaries of very small streams. The components of the external medium are (*a*) stream water, which is rich in Ca and CO_3, and (*b*) Atlantic sea-water. These are mixed in different proportions in different parts of the estuary. An analysis of the stream-water is given. The habitat of the organism is described. This extends roughly from high-water neap tides to low-water neap tides. A faunistic survey is given. The conditions which control the limits of the habitat of Gunda are discussed. Between the upper limit of occurrence of Gunda and the place of occurrence of fresh-water forms there is a region devoid of fauna. This region corresponds roughly with the span between high-water neap tides and high-water spring tides. Salinity determinations have been made on samples taken from the actual places where Gunda occurred. It is shown that Gunda has to withstand changes from completely fresh to undiluted sea-water. It may normally be exposed to either extreme for several hours. Salinity determinations made continually throughout the range of Gunda show that its environment may vary from one in which it is subjected to the action of sea-water for only about one hour at high tide to one in which the sea-water is only diluted to about 10 per cent of its normal strength for a few hours during low tide.

C. F. A. P.

The Adaptation of *Gunda ulvae* to Salinity. II. The Water Exchange.

By E. Weil and C. F. A. Pantin.

Journ. Exp. Biol., Vol. VIII, 1931, *pp.* 73–81.

The effect of fresh waters and dilute solutions on the behaviour and water exchange of the estuarine flatworm, *Gunda ulvæ*, has been studied. In Plymouth tap-water, which contains little dissolved substances, the majority of the worms die within 48 hours. While immersed in this water the worms swell rapidly during the first hour to about double their volume in sea-water, the volume falling slightly after this. The effect is reversible. In dilute sea-water the worms swell to a greater extent the greater the dilution. At great dilutions the swelling is much less than would be expected if the worm behaved as though it were covered with a perfectly semi-permeable membrane. In water from the stream which normally flows over the Gunda at low tide, the swelling of

the worms is much less than in Plymouth tap-water or in distilled water. This stream-water is rich in $CaCO_3$. The effects of distilled water and of solutions of NaCl, $NaHCO_3$, glycerol, $CaCl_2$ and of Cambridge tap-water are compared with the effects of Plymouth tap-water and the stream-water. It is found that the beneficial effects of the stream-water can be imitated only by the solutions containing calcium. The mode of action of calcium is discussed. It is suggested that it acts primarily by lowering the permeability of the worms to water.

C. F. A. P.

The Adaptation of *Gunda ulvae* to Salinity. III. The Electrolyte Exchange.

By C. F. A. Pantin.

Journ. Exp. Biol., Vol. VIII, 1931, *pp.* 82–94.

The rate of loss of salts by the estuarine worm, *Gunda ulvæ*, on transference from sea-water to various dilute solutions has been studied by measurement of the electric conductivity of the surrounding solution. Salts are lost by the worms from the moment of immersion in dilute solutions. Conditions affecting the rate of loss of salts are discussed. The relation between the amount of salts lost and the total electrolyte content of the worm was determined. It is shown that the worms only lose 25 per cent of their salts during the time that they imbibe a volume of water from the dilute solution equal to their initial volume. The limiting internal salt concentration of worms surviving in waters containing calcium is about 6–10 per cent of the normal concentration in sea-water. No such limiting value can be found for distilled water, since salts are lost continuously till cytolysis occurs. The significance of the limiting concentration is discussed. The effect of osmotic pressure, pH, dilute solutions of NaCl, $NaHCO_3$, glycerol, $CaCl_2$ and $CaCO_3$ are studied. The presence of calcium reduces the rate of loss of salts. Other factors do not seem to influence this rate. The relation of calcium to the maintenance of normal permeability to water and salts in the worm, and the significance of this to the problem of migration into fresh water are discussed.

C. F. A. P.

Do Oceanic Plankton Animals lose themselves?

By F. S. Russell.

Nature, January 4th, 1930.

The suggestion is made that there are threshold light intensities below which some plankton animals cease to be stimulated to move upwards. In the open ocean certain plankton animals which live in light of moderate

intensities near the surface may move downwards out of their normal light zone, perhaps at night, and reach layers at which the intensity is below the threshold. There will be no longer any light stimulus to bring them up, and there they may roam until random movement brings them once more into their threshold intensity zone.

F. S. R.

Vitamin Content of Marine Plankton.

By F. S. Russell.

Nature, September 27th, 1930.

Mention is made of the possible connection between the habit of plankton animals in the Plymouth area of living nearer the surface in July and August than in the previous months, and Belloc, Fabre and Simonnet's record that sterols collected from plankton animals in July were found to be biologically active, whereas those collected in April only acquired biological activity after irradiation.

F. S. R.

The Swimming of Cuttlefish.

By F. S. Russell and G. A. Steven.

Nature, June 14th, 1930.

Attention is called to the use made by the cuttlefish of its siphon for slow swimming in all directions, a fact not mentioned in zoological text-books, in which the siphon is only recorded as being used for backward swimming.

F. S. R. AND G. A. S.

Digestive Processes in Marine Invertebrates and Fishes.

By C. M. Yonge.

Jour. du Conseil, VI, 1931, *pp.* 175–212.

This paper contains a summary of recent work on the subject, particular emphasis being laid on the specialization of digestive enzymes characteristic of many groups of Invertebrates, and the gradual development of extracellular digestion in the animal kingdom from the primitive intracellular digestion.

C. M. Y.

The Significance of the Relationship between Corals and Zooxanthellae.

By C. M. Yonge.

Nature, CXXVIII, 1931, *pp.* 309–311.

This is a short summary of the author's papers on this subject published in the Scientific Reports of the Great Barrier Reef Expedition 1928–29 (British Museum (Nat. Hist.)). The conclusion is reached that the association between corals and zooxanthellae is essential to the plants, certainly not to *individual* coral colonies, but probably an indispensable factor in the necessarily exceptional powers of growth and repair possessed by the marine communities known as coral reefs.

C. M. Y.

The Crystalline Style of the Mollusca and a Carnivorous Habit cannot normally co-exist.

By C. M. Yonge.

Nature, Vol. CXXV, 1930, *pp.* 444–445.

Evidence is produced demonstrating that the presence of a crystalline style in any mollusc (Lamellibranch or Gastropod) is a certain indication that the animal in question possesses *no extracellular proteoclastic enzymes* and so cannot digest any but the very minute particles of protein matter which can be digested intracellularly. Such an animal is, therefore, except where a powerful crushing gizzard is present as in the Septibranchs, a specialized herbivore, feeding on phytoplankton or finely divided algal material.

C. M. Y.

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INDEX

A

Abnormal fishes, 53
Acanthias vulgaris, double monster of, 60
Actinia, pigment in, 739–742
Æquorea forskalea, 220, 228
Aglantha, 220, 229, 233, 236
Aglaophenia, pigment in, 743
Aira and soil acidity, 1031
Amœba from intestine of Phallusia, 583
Amphipoda, vertical distribution, 773
Anchialus agilis, vertical distribution, 769, 773
Anemonia sulcata, pigment in, 739, 740
Anguilla vulgaris, changes in length during larval life, 987
Animal tissues, spectrographic analysis, 1038
Antennularia, pigment in, 743
Anthea, pigment in, 740, 741
Apherusa, vertical distribution, 773
Arachnactis albida, 229
— *bournei*, 229
Arenicola ecaudata, 1041
— *marina*, respiration, 709, 724; function of hæmoglobin, 709, 726; habitat and habits, 731
Ascidia aspersa, 2, 4, 10, 19, 31; distribution, 5; eggs, 16; synonymy, 32
— *conchilega*, 2, 15, 22, 29, 1034; eggs, 17; synonymy, 33
— *mammillata*, 2, 24; eggs, 17; synonymy, 33
— *mentula*, 2, 20, 1034; distribution, 20; eggs, 17; synonymy, 33
— *Normani*, 7
— *obliqua*, 3, 15, 25, 31; eggs, 17; synonymy, 33
— *prunum*, 3, 15, 25, 26, 31, 1034; eggs, 17; synonymy, 33
— *scabra*, 2, 4, 10, 15, 19; distribution, 5; eggs, 16; synonymy, 32
— *scabra* var. *albida*, 7
— *scabra* var. *lævis*, 8
— *tritonis*, 28
— *virginea*, 4, 25, 27, 31; eggs, 17; synonymy, 33
Ascidians, identification, 1; physiology, 1034
Ascidiella aspersa, 3, 1034
Ascidiella, cristata, 32
— *scabra*, 3, 1034
— *virginea*, 3
Aurelia, 229; pigment in, 741
Axinus flexuosus, 359

B

Bacteria from bottom deposits in Clyde sea area, 751
Bacterium tumefaciens, radiation hypothesis, 65, 69, 70
Balanus balanoides, 301
Bodotria scorpioides, vertical distribution, 769, 773
Boltenia hirsuta, 1034
Botryllus containing Trivia eggs, 819, 820
Bougainvillia, 220, 224, 234, 770
Boveria stevensi in Galeomma, 577
— *subcylindrica*, 581
— *teredinidi*, 581
Bulla hydatis, 567
Bunodes, pigment in, 741, 742

C

Calanus finmarchicus, 344, 365, 450, 459, 462, 467, 767, 772
Calocaris macandreæ, 351
Candacia armata, vertical distribution, 772
Carassius auratus, perception of electrical currents, 415
Carcinus mœnas, relative growth, 1001
Cardium edule, 278
Chemical and physical conditions of Clyde muds, 326
Chicken, treatment of gapes in, 1033
Chrysaora, pigment in, 741
Ciona intestinalis, 15, 1034
Cletodes longicaudatas, 352
Cliona attacking Mytilus, 518, 521
Clione limacina, 594, 785
Clupea, growth-changes, 997
Clupea pilchardus, growth, 978, 997
— *sprattus*, growth, 980, 997
Cod with malformed mouth, 54
Coelenterate pigments, 739, 740; plankton, 219
Cœlorhynchus cœlorhynchus, gland in, 853; luminescence, 863

Copepods, resistance to anærobic conditions, 356
Corals, feeding and digestion, 599; association between zooxanthellæ and, 1048
Corella parallelogramma, 16
Cosmetira pilosella, 227, 236; vertical distribution, 771
Crab nerve, carbohydrates of, 1041
Crustacea, chitinous membrane, 600; vertical distribution of larvæ, 773–775
Cumacea, vertical distribution, 769, 773
Cuttlefish, swimming, 1047
Cyanea, pigment in, 741
Cyanea lamarcki in Plymouth area, 573
Cynosurus cristatus and soil acidity, 1030

D

Dactylis glomerata and soil acidity, 1031
Danielssenia typica, 352
Dendrodoa grossularia, 15
Deterioration of exposed fabrics, 473
Diastylis rostrata, vertical distribution, 769, 773
Diatoms, in Clyde sea area mud, 343; utilisation of salts by, 596; growth under light filters, 599; food for larval molluscs, 800
Dicystid gregarines, 1042
Diplosoma listerianum var. *gelatinosum*, food of Trivia, 819, 820
Dipurena (*Slabberia*) *halterata*, 223
Dodecaceria concharum in Mytilus, 521
Drift bottle experiments, 243

E

Echinus miliaris and mitogenetic radiation, 70; effect of severe winter, 301, 308
Eel, changes in length, 995
Ensis siliqua, 1039
Entamœba phallusiæ, n. sp., 585
Euphysa, 220
— *aurata*, 223, 234
Eurypon clavatum, 839, 842
Eutonina indicans, 220, 228, 236

F

Fæcal pellets in Clyde sea area, identification, 359
Filograna implexa, 1037
Fishes, abnormalities, 53; perception of electrical currents, 415; schooling, 421; death from pollution, 597; luminescence, 853; digestive processes, 1047
Fucus vesiculosus as food for Ligia, 657, 663, 675

G

Gadus callarias, perception of electrical currents, 416
Galathea dispersa, 175; larvæ, 180, 385, 388
Galathea intermedia, 175, 177, 181; larvæ, 385, 387
— *nexa*, 175, 385
— *squamifera*, 175; larvæ, 385, 386, 388
— *strigosa*, 175; larvæ, 180, 388
Galatheidæ, larvæ of Plymouth, 175, 385
Galeomma turtoni, 577
Gammarus chevreuxi, eye colour changes, 189, 593
Gasterosteus aculeatus, schooling habit, 422
Gasterostomum infesting Mytilus, 522
Gellius angulatus, 839, 841
Gobius ruthensparii, 422
Gonospora (*Kalpidorhynchus*) *arenicolæ*, 1041
Gunda ulvæ, adaptation to salinity, 1045

H

Halecium, pigment in, 743
Halichondria firmus, 839, 842
Harpacticus uniremis, 352
Hedera helix, 1030
Heliopora cærulea, pigment in, 740, 741
Hentschelis, n. gen., 1042
Herring, biology, 591; pug-headed, 55
Hybocodon prolifer, 220, 224, 233, 234
Hydrogen ion concentration, 1037; geochemical applications, 1029
Hydroid pigments, 739
Hyperidion thalassemæ n. gen., n. sp., 1042

I

Ilex aquifolium, 1030

L

Labyrinthomyxa, 837
Labyrinthula, 833
Lamellaria perspicua, 819, 822
Larix europœa, 1030
Lecythion, n. gen., 1042
Leptomysis gracilis, vertical distribution, 769, 773
Leuckartiara, vertical distribution, 769
Leuckartiara octona, 220, 226; vertical distribution, 771
Ligia oceanica, habitat, 656; change of environment, 656; effect of temperature, 659; moulting and breeding, 663; food and feeding, 675; structure of foregut, 676
Limacina retroversa, 594
Lizzia blondina, 220, 225, 234
Lolium perenne and soil acidity, 1030

M

Macoma baltica, 296
Macroplankton, vertical distribution, 391, 767
Malacocephalus lævis, 853, 861, luminescence, 863
Maldanids, fæcal pellets, 364

Marine aquarium, condition of water, 479, 1037
Marine invertebrates, digestive processes, 1047
Medicago sativa and soil acidity, 1031
Meganyctiphanes norvegica, fæcal pellets, 365
Mitogenetic radiation, 65
Mitrocoma (*Mitrocomella*) *fulva*, 220, 226, 234
— *polydiademata*, 226
Modiolus modiolus, ciliary movement, 537
Molgulidæ, abbreviation of development, 1035
Mollusca, crystalline style, 1048
Morone labrax, schooling habit, 422, 425, 593
Mud bacteria, 758
Muds of Clyde sea area, 325, 751
Munida banffica larvæ, 175, 179, 181
— *rugosa*, 179
Murex erinaceus, 301, 305
Mytilus edulis, abnormal gills, 489; labial palps, 545; foot, 548; regeneration of gill, 551; food for plaice, 878

N

Nardus stricta and soil acidity, 1031
Nassa incrassata, 594, 797
Nassarius incrassatus, larval stages, 797, 804
— *reticulatus*, larval stages, 797, 803
Nematodes, resistance to anærobic conditions, 356
Nitzschia closterium, 596; effect of light on growth, 633; food for larval molluscs, 801
Nucella (=*Purpura*) *lapillus*, 1043
Nucula nitida, 361
— *nucleus*, 361
— *radiata*, 361
— *sulcata*, 362
— *tenuis*, 356, 361
Nyctiphanes couchii, fæcal pellets, 365

O

Obelia, 220, 227, 236; vertical distribution, 771
Ocenebra erinacea, 1043
Oikopleura, in vertical hauls, 450
Ostrea edulis, sex-change, 315
Owenia fusiformis, 595
Oyster drills, effect of severe winter, 301; in Essex estuaries, 1043
Oysters, experimental breeding, 596; giant, 1043; spawning, 1044

P

Pachymatisma johnstoni, 839
Pandalina brevirostris, 767; vertical distribution, 769
Paracalanus parvus, 450, 462
Paracentrotus lividus and mitogenetic radiation, 66
Pecten, adductor mechanism, 1033, 1036; style enzyme, 1040
Pecten septemradiatus, 363
Phallusia, 30
— *cristata*, 32
— *mamillata* containing amœbæ, 583; development, 1034
Phialidium, 220, 228, 236; vertical distribution, 771
Photo-electric photometry, 598, 618, 1030, 1032; apparatus for, 1017, 1022; recording of daylight, 1029–1033
Phytoplankton, seasonal changes, 599
Picea excelsa, 1030
Pilchard, fan-tailed, 56; growth in length, 977
Pinnotheres in Mytilus, 490, 520, 523; relative growth, 1012
Plaice, absorption of fats and lipoids, 75; growth and maintenance, 103, 877, 944
Plankton, of Northumbrian coast, 219, 233; validity of single vertical hauls, 449; light stimulus, 1046; vitamin content, 1047
Planorbis corneus, respiration, 709; function of hæmoglobin in, 709, 714
Plant distribution in relation to illumination, 1030; in relation to soil acidity, 1030
Pleuronectes microcephalus, coalescence of vertebræ in, 57
— *platessa*, 75, 103
Podocoryne areolata, 224
Polyclinum luteum containing Trivia eggs, 819
Polydora infesting Mytilus, 518, 520–522; containing dicystids, 1042
Polyrhabdina, 1042
Pomatoceros triqueter, tube formation, 379
Portunus depurator, 767
Potentiometer, 1018
Protodrilus flavocapitatus, 1039
Psammechinus miliaris, regeneration of spines, 1041
Pseudaxinella alleni n. sp., 839, 843
— *egregia*, 843
— *sulcata*, 843
Pseudocalanus elongatus, 450, 462
Pteris aquilinum, 1030
Purena (*Sarsia*) *gemmifera*, 220, 223
Purpura lapillus, 301

Q

Quercus ilex, 1030

R

Raia batis, 368, 369
— *brachyura*, 368, 370, 375
— *circularis*, 368, 370, 373
— *clavata*, 368, 370, 375, 592; abnormal, 59
— *fullonica*, 368, 370

Raia marginata, 368, 370
— *microcellata*, 368, 370, 377; abnormal, 59
— *montagui*, 368, 370, 375
— *nævus*, 368, 370, 373
— *oxyrhynchus*, 368, 369
— *undulata*, 368, 370, 373
Rathkea octopunctata, 220, 225, 234
Rays, abnormal, 59; method of rapid identification, 367
Reniera aquæductus, 839
Report of the Council, **1930**, 587
Respiratory pigments affected by potassium, 1037
Rhizostoma cuveri, pigment in, 739
Rissoa, 594
River Tees survey, 597
Rubus fruticosus, 1030

S

Sabella pavonina, regeneration, 1035; feeding mechanism, 1042
Sagartia, pigment in, 741
Sagitta, behaviour of, in Plymouth area, 391; vertical distribution, 450, 770, 772
Sagitta bipunctata, 392, 403
— *elegans*, 392, 396
— *serratodentata*, 595
— *setosa*, 392, 396
Saphenia gracilis, 228; vertical distribution, 772
Sarsia eximia, 222, 233
— *prolifera*, 220, 222
— *tubulosa*, 220, 233
Scatella stagnalis, early stages, 847
Scololepis fuliginosa containing dicystids, 1042
Sea-urchins, regeneration of spines, 1041
Seawater, colorimetric determination of pH, 483; salinity, 596, 599
Sertularella, pigment in, 743, 744
Sertularia, pigment in, 743
Sex-change in European oyster, 315
Skates, methods of rapid identification, 367
Skeletonema costatum, 343, 800
Spinachia vulgaris, deformed, 59
Sponge fauna of Plymouth, 839
Sprat, growth in length, 977
Steenstrupia, vertical distribution, 770, 771
Steenstrupia (*Corymorpha*) *nutans*, 224
Stentor, pigment in, 741
Styelopsis grossularia, 1034
Submarine illumination, apparatus for measurement of, 1017
Syndosmya alba, 355, 356, 359, 363
— *nitida*, 359, 363

T

Tellina tenuis, 278, 291
Temora longicornis, 450, 462, 464
Tethyum pyriforme, 1034
Thalassema neptuni, containing gregarines, 1042
Thalassiosira gravida, food for larval molluscs, 801
Themisto gracilipes, vertical distribution, 770, 773
Thuiaria articulata, pigment in, 744
Thysancessa raschii, fæcal pellets, 365
Tiaropsis multicirrata, 227, 236
Tima bairdii, 228, 236
Tomopteris, vertical distribution, 770, 772
Trididemnum tenerum, food for Trivia, 819
Trifolium and soil acidity, 1031
Trivia europea, larval stages, 819
Tunicate development, 1034, 1035
Turris pileata, 771

U

Uca, relative growth, 1011
Urosalpinx cinerea, 301, 305, 1043

V

Velella spirans, pigment in, 740

W

Water movements in English Channel, 241

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